

STUDIES OF
DEVELOPMENTAL ANOMALIES
IN THE DESCENDANTS OF
X-RAYED MICE

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF
SCIENCE IN THE UNIVERSITY OF MICHIGAN

[Reprinted from PAPERS OF THE MICHIGAN ACADEMY OF SCIENCE,
ARTS AND LETTERS, Vol. X, 1928. Published 1929.]

Ann Arbor 1929

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PART I: GENETIC BEHAVIOR

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INTRODUCTION

DURING 1921, Drs. C. C. Little and H. J. Bagg, in an attempt to change the character of the germ-plasm in a stock of colored mice, treated a selected group of these animals with a light dose of X-rays. The early results of these irradiations were reported by these co-authors in their papers of 1923 and 1924. In these reports they described four structural defects which were apparently caused by the treatment of X-rays:

1. An eye abnormality;
2. An abnormality of the pigment and hair of the back which they called "saddled";

3. A kidney abnormality (Bagg, 1923);
4. An abnormality of the feet.

Part I of the present paper is an attempt to describe the form and inheritance of the foot abnormality, which was first reported by the original workers. Part II is the record of a study made to ascertain whether observable structural changes in the motor mechanism of the spinal cord could be correlated with the foot abnormalities. Brief reference will also be made to eye and kidney abnormalities.

The author is greatly indebted to Drs. C. C. Little and G. Carl Huber for their untiring help and encouragement in the preparation of this work as a whole; and to Dr. R. E. McCotter for his advice in making the cell counts, to Dr. P. S. McKibben for his help with the photography, and to Miss Elizabeth Fekete for her sketches of the abnormalities.

HISTORICAL

Since the theory of pangens was first set forth by Darwin, the literature is replete with records of attempts to change the germ-plasm. The agents used in these efforts have been many and varied, ranging from specific mutilations — Weismann (1889), Charrin (1902), Delage¹ (1903) — to changes in environment and the use of chemical and physical agents. The methods of administering these treatments have been as numerous and diverse as have been the types of treatment themselves.

Since the rebirth of Mendelism in 1900, Lamarckism suffered a relapse until Guyer and Smith (1918, 1920, 1920 a, 1921, 1922, 1922 a, 1924), Stockard (1913, 1916, 1921, 1923, 1923 a, 1924), Stockard and Craig (1912), and Stockard and Papanicolaou, with their work on lens antibodies and alcohol, respectively (1916, 1918), brought up the question anew.

Guyer and Smith contend that by introducing a specific antibody (lens protein of the rabbit) into the blood of a fowl, they are able to bring about changes in the fowl which encourage the building up of antibodies which have a tendency to protect the birds

¹ A complete history of the early theories is given by Delage, Y., Paris, 1903, *L'Hérédité et les grands Problèmes de la Biologie Générale*.

against the foreign proteins. If, then, this antibody is taken from the fowl and introduced into the blood-stream of a pregnant rabbit, it appears to act directly upon the optic lenses of the developing fetuses and causes them to have abnormal eyes. In addition to this, Guyer and Smith state that these antibodies evidently have a direct effect upon the germ-plasm of the embryos in that the same deformity which occurs in the embryos of the treated rabbits is handed down to their progeny as a Mendelian recessive. Stockard finds much the same sort of deformity in the young of guinea pigs which were subjected to alcohol fumes for long periods. He also claims that the young of alcoholized males have abnormal eyes and that the germ cells are affected, inasmuch as these abnormal guinea pigs pass on this abnormality to their young.

The most popular of the physical agents that have been used in attempts to change the germ-plasm have been radium emanation and X-rays. These agents have been used largely because of ease of application and the very definite abnormalities which they are capable of producing. They also have interesting bearings upon therapeutic methods in medicine.

The early work with radio-active substances was correlated with the production of parthenogenesis and monstrosities. G. Hertwig (1913) found that by treating the sperm of *Rana fusca* from two to three hours between capsules of mesothorium, he could obtain the same results in the eggs fertilized by the radiumized sperm as Bataillon (1910) had obtained by sticking the mature ovum of the frog with a fine platinum needle. In both cases the egg developed normally and gave rise to swimming tadpoles. O. Hertwig (1913), repeating the work of his son upon the eggs and sperm of *Triton vulgaris* and *Salamandra maculata*, showed that many of the eggs failed to become fertilized; some showed polyspermy and only a few developed normally. He concluded from the data that the chromatin material of the sperm had been broken down by the effect of the radium, and that the same condition was obtained in the pronucleus of the treated eggs. The work of P. Hertwig has also been outstanding in this field. These findings of the Hertwigs bore out the observations of many earlier work-

ers — Bohn (1903), Danysz (1903), Perthes (1904), Bordier (1905), and Thies (1905) — all of whom had reported abnormal development and monstrosities in various forms treated with radium and X-rays. Later, Tur (1911), Lacassagne and Coutard (1923), and Baldwin (1919) reported similar deformities in the embryos of birds and the larvae of frogs treated with X-rays. In the same year, 1911, Loeb and Bancroft (1911) reported that they had obtained several mutants in *Drosophila* which had been treated with X-rays. Later, Bagg (1920, 1922) found that in mammals radium emanation had a selective action upon fast-growing tissues, and that it caused very decided developmental arrests in the nervous and reproductive systems of animals exposed to the rays near the end of pregnancy. He found that radiation produced extravasations of blood in the subcutaneous tissues of the developing embryos and that the radium emanation possibly had a specific effect upon endothelial tissues lining the blood vessels. Bailey and Bagg (1923) found that radiation before fertilization disturbed and arrested the development of mammals, leading to the formation of monsters which conform to a general type. In these experiments they found a pronounced disturbance in the central nervous system and a marked tendency toward progressive loss of fertility, thus confirming the work of Bohn, Perthes, the Hertwigs, Tur, Bordier, and Baldwin. They also ascertained that irradiation during pregnancy caused disturbed abnormal development, absorption, sterility, and lesions of the central nervous system, as well as blood vascular disturbances in the embryos. These observations were also made by Hippel and Pagenstecher (1907), Regaud, Nogier, and Lacassagne (1912), Lengfellner (1906), Krukenberg (1909), Cohn (1909), Bagg (1922), Hanson (1923), Lacassagne and Coutard (1923).

Mavor (1921, 1921 a, 1922, 1923, 1923 a), after treating *Drosophila melanogaster* with X-ray irradiation, found that it might cause non-disjunction of the "X" chromosomes during either the first or the second maturation division, producing in this way by non-disjunction "exceptional" males and females in greater number than ordinarily occurs among breeding animals. This work was later confirmed by Anderson (1924).

Bagg (1920) found that repeated injections of radio-active salts into rats brought on marked changes in the content of the blood-stream, together with fatty degeneration of the liver and hemorrhage of all organs.

More recently, Muller (1927), working with *Drosophila*, found that "the general frequency of gene mutation may be raised at will to practically any desired extent" by X-rays; and that "re-arrangements in the order of the genes may be produced in abundance by X-rays."

It is seen from these reports that radium emanation and X-ray irradiation caused marked changes in the living organisms, affecting both the soma and the germ-plasm.

ORIGIN OF THE STOCK USED

The animals used in this experiment are the descendants of mice X-rayed by Little and Bagg, and which were reported by them in their communications of 1923 and 1924. These mice were selected from a stock of colored animals which had been inbred for several years by Miss A. E. C. Lathrop and her successor, Mrs. Gray. The latter has stated that at no time have eye or foot abnormalities of the type described in this paper, or of any similar type, appeared in this stock.

CONDITIONS OF EXPOSURE TO X-RAYS

In the original experiment of Little and Bagg, animals of both sexes were separated into random groups, one group being X-rayed and the other being held as controls. The X-rayed animals (all of which were adults when irradiated), twenty males and ten females, were treated in the following manner:

The physical conditions were —

1. Target skin distance, 12 inches.
2. Spark gap, two and one half inches.
3. Ten milliamperes of current.
4. No filter.
5. Twelve-second exposures were given upon five successive days.

6. The mice were confined in a small space directly beneath the X-ray tube. The entire dorsal surface of the body was exposed.

Except for a ten-week period of sterility, no immediate physiological effects were observed to follow the treatment.

Of the 20 males, 14 died or were sterile and gave no young. Of the 10 females, 3 died without leaving descendants, 3 were mated with 3 different males, and the other 4 were divided into 2 groups of 2 with a treated male for each group. Two treated males were mated with untreated females. Of these matings, female 85, mated to male 49 (both treated animals), gave 3 normal young. These were bred *inter se* and gave 6 young which were themselves inbred brother to sister. Among the offspring, a single mouse (σ 1369) was observed to have an abnormal eye. After the appearance of this abnormal male, 16 abnormal young were obtained from this generation.

The same abnormalities were found in the third generation of a different pair (φ 86 $\times$ σ 36) of X-rayed individuals.

No abnormalities occurred in over 2,000 control animals.²

Pedigree Charts I, II, and III (Figs. 41-43) show the lines of descent of many of the stock animals. The numbers to the left of the vertical lines are the ledger numbers of the females involved in the crosses; the numbers to the right of the vertical lines are the ledger numbers of the males to which they were bred. Underneath the ledger numbers are written the descriptions of the abnormalities of the animal bearing the ledger number above it. To the right of the ledger numbers of each mating is written a fraction, the numerator of which represents the number of abnormal young produced by this pair, while the denominator represents the total number of young born to this mating. The following symbols are used to describe the deformities of the abnormal animals:

1. *hh* for the head or eye abnormality.
2. *ff* for the foot deformity.
3. *s/sl* for the saddled condition of the back.

² Since that time several thousand additional animals have been produced by this stock. All are normal.

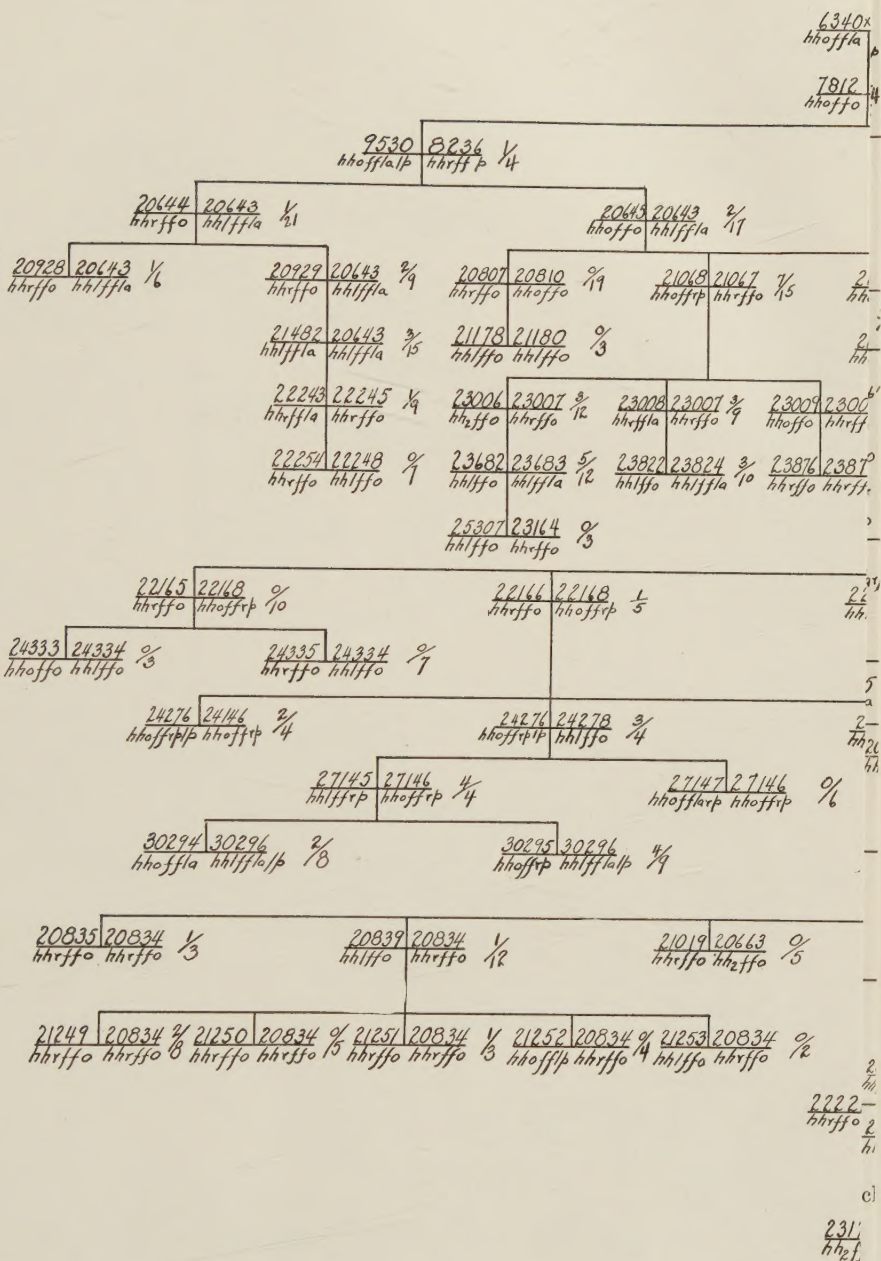


FIG. 42. Pec

6339 $\frac{5}{11}$
hhrrffa

7816
h h o f f r a l a $\frac{2}{5}$

9532	8236	%
hhoffra	hhrrffp	

$\frac{20570}{hhoffo} \mid \frac{20569}{hhoffo} \frac{9}{5}$

20568	20569	3
hhofo	hhofo	20

20252	20254	8/
hhvffo	hhoffo	21

1121	20595	2
2ffo	hhffra	3

20735	20734	2/8
hh/ffo	hhoffo	

20736	20734	$\frac{2}{5}$
h h o f f o	h h o f f o	

20662	20663	$\frac{9}{14}$
hh r t t o	h h ₂ t t o	

1960	21961	2/6
ffffp	hhrfffralp	

21016 | 21015 $\frac{1}{5}$
hhrrffo hhlffo

22176	21682	%
hhoffrp	hhfffo	4

$1\frac{2}{17}$

$\frac{22179}{hhoffo} \mid \frac{21682}{hh\check{v}ffo} \quad \frac{1}{12}$

$7\frac{6}{10}$

$\frac{167}{160} \frac{22168}{h h o f f r p} \%$

$$\frac{4279}{rffo} \mid \frac{24278}{hhfffo} \quad \frac{2}{3}$$

21020	20663	2
hh/ffo	hh ₂ ffo	14

hh ₂ ffo	hh ₂ ffo	%
21021	20663	

$$\frac{21022}{hh_1ffo} \mid \frac{2066.3}{hh_2ffo} \%$$

21526	21525
hh ₂ ffo	hh ₁ ffo

 $\frac{1}{7}$

$$\begin{array}{r} 2 \overline{) 22220} \\ \underline{hhfflq} \end{array} \quad \frac{1}{5}$$

22223	22220	%
hhoffo	hh/ffa	

22414	22413	%
hhfffo	hhfffo	

$\frac{22415}{hhoffo} \quad \frac{22413}{hhloffo} \quad \frac{2}{12}$

77	23179	%
fo	hh ₂ ffo	14

23748	23749	0/
hhooffo	hhooffo	17

23455	23456	9
hhoffo	hhloffo	11

23457 23456 $\frac{1}{13}$
hhfffo hhlffo

$$\begin{array}{r|l} 23458 & 23456 \\ \hline h h_2 f f o & h h l f f o \end{array} \quad \frac{9}{3}$$

ligree chart II

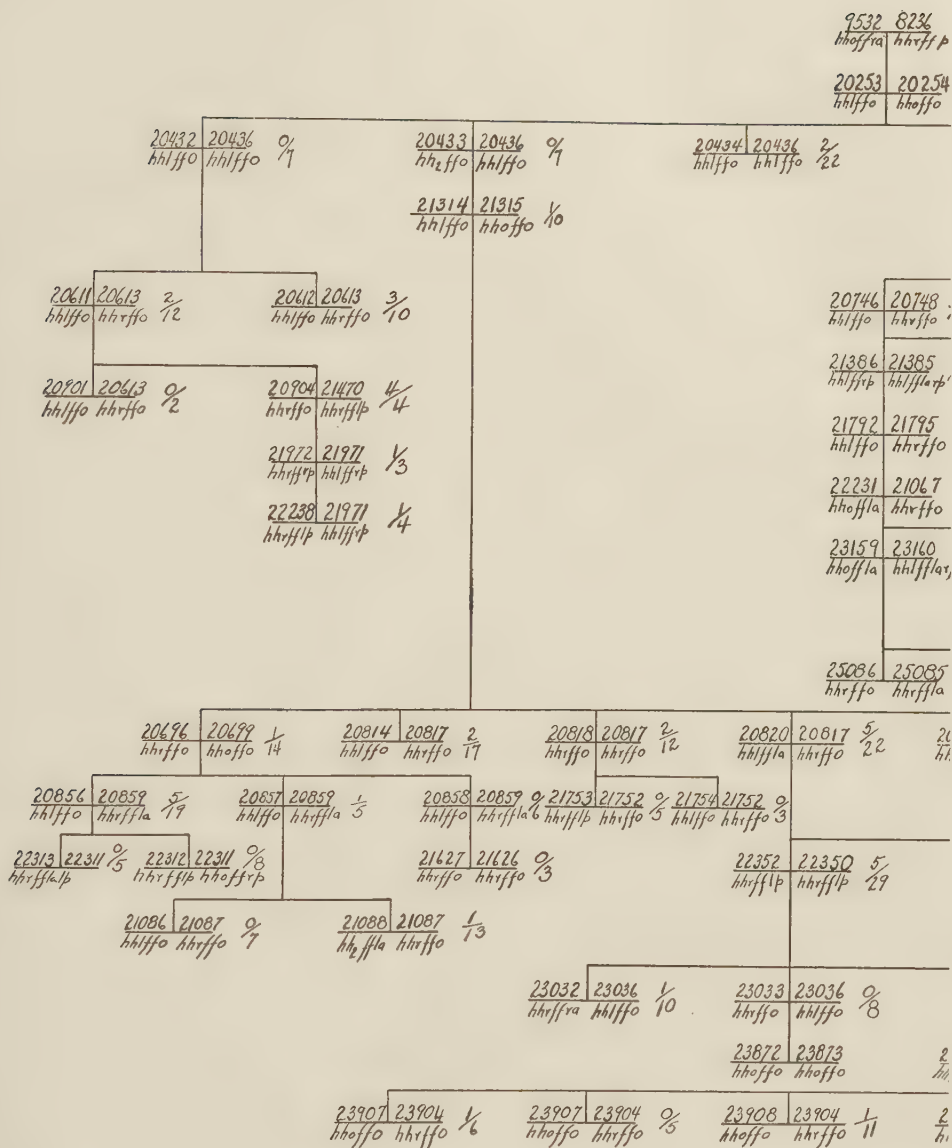


FIG. 43. Pedigree c)

$\frac{2}{7}$

$\frac{6}{20}$

$\frac{20435}{hhffo}$	$\frac{20436}{hhffo}$	$\frac{2}{11}$	$\frac{20524}{hhffo}$	$\frac{20525}{hhffo}$	$\frac{4}{13}$	$\frac{20962}{hhffo}$	$\frac{20965}{hhffo}$	$\frac{1}{8}$
$\frac{20727}{hhffo}$	$\frac{20728}{hhffo}$	$\frac{2}{13}$				$\frac{21645}{hhffo}$	$\frac{20965}{hhffo}$	$\frac{2}{11}$
$\frac{21065}{hhffo}$	$\frac{21062}{hhffo}$	$\frac{1}{2}$	$\frac{21066}{hhffo}$	$\frac{21062}{hhffo}$	$\frac{1}{4}$	$\frac{22194}{hhffo}$	$\frac{22196}{hhffo}$	$\frac{2}{11}$
$\frac{21386}{hhffo}$	$\frac{21795}{hhffo}$	$\frac{3}{6}$	$\frac{21508}{hhffo}$	$\frac{21504}{hhffo}$	$\frac{2}{6}$	$\frac{22492}{hhffo}$	$\frac{22493}{hhffo}$	$\frac{2}{16}$
						$\frac{22492}{hhffo}$	$\frac{22492}{hhffo}$	$\frac{1}{18}$
						$\frac{23625}{hhffo}$	$\frac{23624}{hhffo}$	$\frac{2}{18}$
						$\frac{23686}{hhffo}$	$\frac{23688}{hhffo}$	$\frac{2}{11}$

$\frac{23161}{hhffo}$	$\frac{23160}{hhffo}$	$\frac{2}{19}$	$\frac{23163}{hhffo}$	$\frac{23160}{hhffo}$	$\frac{2}{15}$
$\frac{24767}{hhffo}$	$\frac{24765}{hhffo}$	$\frac{3}{8}$	$\frac{24768}{hhffo}$	$\frac{24765}{hhffo}$	$\frac{2}{7}$
$\frac{25084}{hhffo}$	$\frac{25085}{hhffo}$	$\frac{1}{6}$			

$\frac{20942}{hhffo}$	$\frac{20941}{hhffo}$	$\frac{2}{8}$	$\frac{20943}{hhffo}$	$\frac{20941}{hhffo}$	$\frac{1}{11}$	$\frac{21079}{hhffo}$	$\frac{21080}{hhffo}$	$\frac{2}{3}$	$\frac{21082}{hhffo}$	$\frac{21080}{hhffo}$	$\frac{2}{5}$
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$\frac{22353}{hhffo}$	$\frac{22350}{hhffo}$	$\frac{2}{18}$
$\frac{23614}{hhffo}$	$\frac{23613}{hhffo}$	$\frac{1}{12}$

			$\frac{23476}{hhffo}$	$\frac{23471}{hhffo}$	$\frac{3}{5}$	$\frac{25510}{hhffo}$	$\frac{25513}{hhffo}$	$\frac{1}{8}$			
3035	$\frac{232036}{hhffo}$	$\frac{1}{21}$	$\frac{24716}{hhffo}$	$\frac{24718}{hhffo}$	$\frac{2}{6}$	$\frac{24717}{hhffo}$	$\frac{24718}{hhffo}$	$\frac{2}{6}$	$\frac{24818}{hhffo}$	$\frac{24819}{hhffo}$	$\frac{4}{20}$
2960	$\frac{23957}{hhffo}$	$\frac{2}{16}$									

Chart III

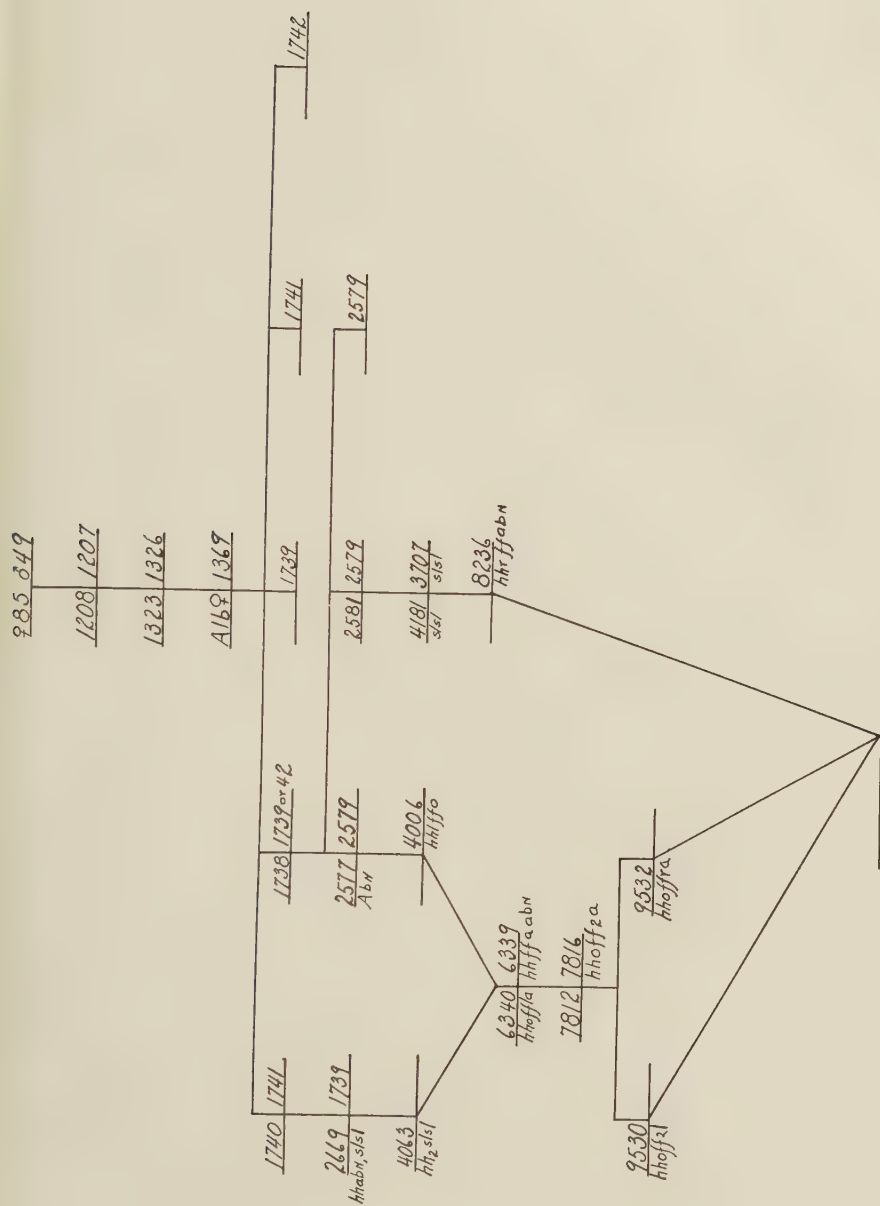


FIG. 41. Pedigree chart I

In this manner, *hhrffralp* is used to designate an animal which was abnormal in its right eye as well as in its right anterior and left posterior feet.

Chart I (Fig. 41) shows the lines of descent of the animals used in the present experiment. Starting with the mating ♀85 × ♂49, the animals were inbred for two generations, producing as an F₃ animal abnormal male 1369. This male, outcrossed to a normal albino female of the Bagg strain, produced 5 young — 2 females and 3 males, all of which were normal. F₁ female 1740 of this litter mated to her brother 1741 gave, among others, female 2669, which was abnormal-eyed. Mated to the sibling of her parents (♂1739) she produced female 4063 *hh₂slsl*. The second female (♀1738) of the original outcross mating (alb. ♀ × ♂1369) bred to her brother (either ♂1739 or ♂1742) produced 2 females and 1 male. The first of these females (♀2577), mated to her brother (♂2579), gave male 4006, which had an abnormal left eye. This male (♂4006) mated to female (♀4063) mentioned above, gave 2 abnormal young — female 6340 which was abnormal in its left foreleg, and male 6339 which also had an abnormal front leg. These two animals (♀6340 *hhoffla* × ♂6339 *hhoffabn*) gave female 7812, which was somatically normal, and male 7816, which was abnormal in both front feet. These two animals when mated (♀7812 *hhoffo* × ♂7816 *hhoff₂a*)³ gave two females — female 9530 *hhoff₂l* and female 9532 *hhoffra*.

Another daughter of the mating (♀1738 × ♂1739 or ♂1742) namely, female 2581, mated to her brother 2579, produced in different litters female 4181 and male 3707. Both these animals were saddled. Mated to each other, they produced male 8236 which had an abnormal foot. This male, bred to the two sisters (♀9530 *hhofflalp* and ♀9532 *hhoffra*) mentioned above, was the starting point of the stocks from which the data of the present computations were compiled.

Turning to Pedigree Charts II and III (Figs. 42–43), we are able to trace the lines of descent of the young of male 1369 and the normal Bagg female through fifteen generations. For the most part, this descent has been through brother to sister mating

³ In certain of the earlier records *ff₂a* is used interchangeably with *fflara*.

(in a number of cases, females have been bred to their father, and in some instances a male has been used which was not as closely related as a brother). All the animals used, however, were the descendants of male 1369 through the cross to the normal Bagg females.

DESCRIPTION OF THE FOOT ABNORMALITIES

Figures 44-46 show drawings of adult mice of the stock first described by Little and Bagg (1923, 1924). These deformities originally appeared in a colored race of mice, but have since been extracted and are now carried by albinos.

Figure 44 shows a left eye which is only slightly abnormal, the deformity being apparent only in the enlarged pigmentation⁴ of the lids. The left front foot indicates one of the more extreme types of deformity. All the bones are reduced, the metacarpals and phalanges being much shortened. The left hind foot demonstrates much the same type of deformity, the remnants of the long bones of the foot being flexed dorsally in a marked degree. The drawing exaggerates to some extent the deformity of the leg, but there was a marked atrophy in the region proximal to the ankle joint.

Figure 45 shows another mouse of this same stock. The right eye of this animal is more markedly abnormal than the left eye of the mouse shown in Figure 44. The right front foot in this case demonstrates the slight shortening of the bones which sometimes occurs. The bones of the right hind foot are seen to be grossly abnormal. This mouse also demonstrates the deformity of the head which sometimes accompanies this eye defect. When compared with the head of the mouse in Figure 44, it is seen to be longer and the frontal portion much flatter.

Figure 46 shows a still greater atrophy of the right eye, and a shortening of the bones of the right anterior foot. Here, as in Figure 45, the foot is apparently not distorted by twisting.

⁴ It is interesting to note that, although the animals are albinos, in some cases of abnormality, pigment, possibly pathological in nature, may be formed at or near the eye.

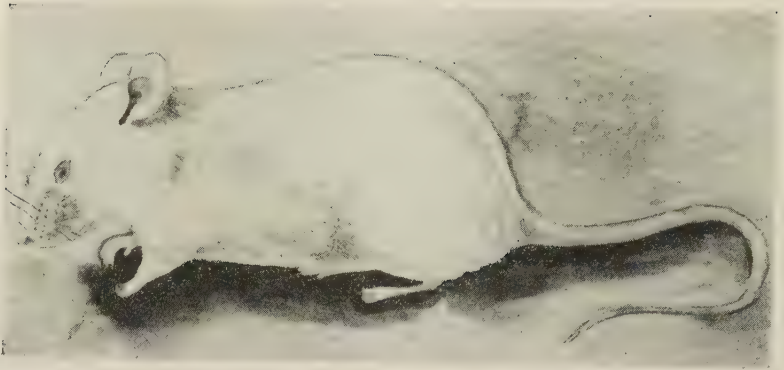


FIG. 44

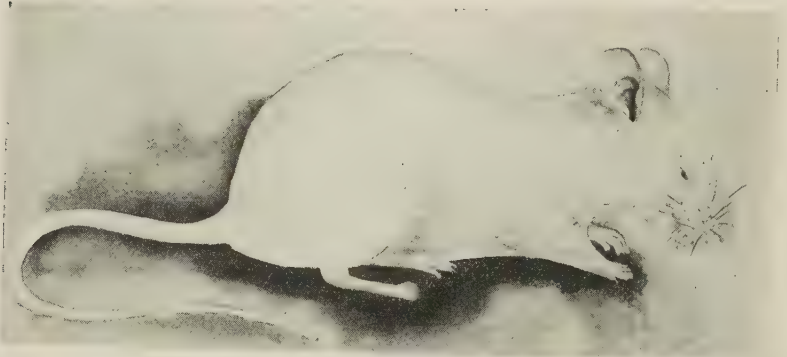


FIG. 45

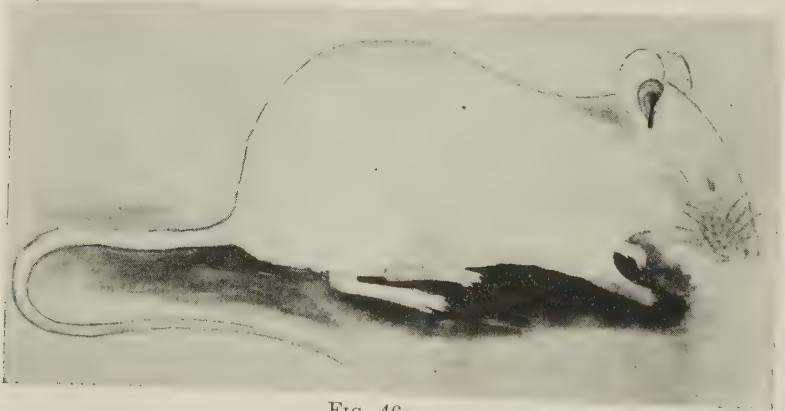


FIG. 46

FIGS. 44-46. Drawings showing variations in the abnormalities of the eyes and the feet

THE NORMAL FOREFOOT

In the normal anterior foot (Figs. 47₁ and 49₁) there are seen to be eight bones in the carpus, five metacarpals, five first phalanges, four second phalanges, and five third phalanges. The thumb or first digit is seen to be very short, the second phalanx is absent, and the distal phalanx extends but a short distance beyond the middle of the metacarpal bone of the index digit. On the thumb there is no nail or claw. The index digit is much



FIG. 47. Drawings comparing abnormal with normal feet

longer than the thumb, the distal phalanx extending well beyond the middle of the proximal phalanx of the second or middle finger. The musculature between the index and second digits extends well down until it is proximal to the metacarpal phalangeal joint of the second finger. The second and third digits are of approximately the same length, the musculature between them extending to a point just proximal to the metacarpal phalangeal articulation. The fourth or little finger is the shortest of all the digits save the thumb, extending about half-way up the proximal

phalanx of the third finger. All four digits bear nails or claws and when in the relaxed state are approximately straight, the bones all lying in the same plane horizontally.

ABNORMALITIES OF THE FOREFOOT

The abnormalities of the front foot vary through a wide range of distortion, extending from slight twists in a single component of one of the digits to a fusion of all the bones and torsions in all directions.



FIG. 48. Types of abnormal feet

Figure 47₃, which is a drawing of the right anterior foot of mouse 1609, shows the thumb to be in approximately the correct position, but smaller than the normal thumb. The four fingers are seen to be very short, the middle digits being twisted toward the thumb. The musculature extends very nearly to the end of the distal phalanges. The little finger, instead of being set at an angle to the third digit, is drawn in very close to it; the musculature and skin which join the two extend clear to the nail on the proximal side. All four of the nails are more or less

contorted and twisted, that of the little finger being the most disfigured.

Figure 47₄, the left anterior foot of mouse 1515, is of approximately the same type as the foot shown in Figure 44, the middle fingers being apparently fused. Figures 47₅ (right anterior foot of mouse 1617), 47₆ (right anterior foot of mouse 1308), 47₇ (left anterior foot of mouse 1617), 47₈ (left anterior foot of mouse 1235), 47₉ (right anterior foot of mouse 1507), 47₁₀ (left anterior foot of mouse 1507); 48₁ (right anterior foot of mouse 1718), and 48₂ (left anterior foot of mouse 1514) all show this same general type of abnormality. Figure 48_{1,2} shows, in addition to the lateral and medial tortions, a dorsal bending as though pulled by the extensor muscles.

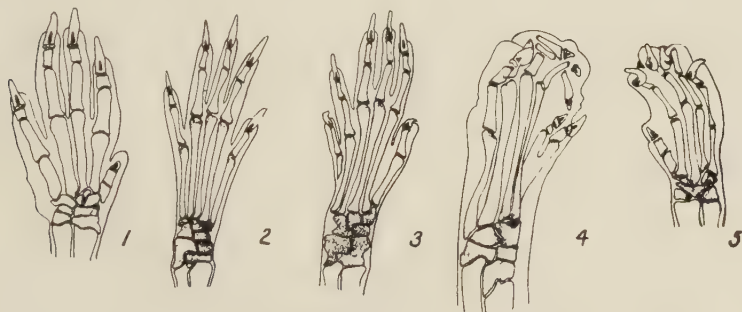


FIG. 49. Variations in bony structure

More deformities of the extremities are shown in Figure 48₂₋₁₁. In Figure 48₃ (left anterior foot of mouse 1618) the thumb has almost entirely disappeared, while the index and second digits are bent sharply toward the middle line. The third and fourth digits are of nearly the same length and diverge markedly from each other. In Figure 48₄ (left anterior foot of mouse 1684) the palmar pad extends in a proximal direction well beyond the base of the thumb, and the four fingers extend radially from a common center. All four fingers show a slight dorsal flexion. Figure 48₅ (left anterior foot of mouse 1340) shows a marked atrophy of all the bony elements of the foot, fusions of all the digits and a sharp flexion. Figure 48₆ (left anterior foot

of mouse 1513) shows even greater atrophy than does Figure 48₂. Here also the dorsal flexion is very sharp. Figure 48₇ (right anterior foot of mouse 1513) is of the same general type as those first described except that the dorsal flexion is most extreme, the bones being bent dorsally so sharply that the nails appear to grow from the back of the foot. Figure 48₈ (right anterior foot of mouse 1507) shows a case of polydactylism.

THE NORMAL HIND FOOT

In the normal hind foot (Figs. 47₂ and 49₂) there are seven bones in the tarsus, five metatarsals — the middle three of approximately the same length and the two outside bones extending well beyond the middle of the metatarsals of the first and third toes. The middle phalanx is missing from the great toe, but is present in all of the digits. The three middle digits are of about the same length; the musculature between them extends almost to the region of metatarsal phalangeal articulation. The great toe and the little toe are set at acute angles to the other toes, the musculature extending to the metatarsal phalangeal joint in each instance.

ABNORMALITIES OF THE HIND FOOT

Abnormalities of the hind foot vary over a wide range, as do those of the front foot. Figure 48_{9, 10, 11} reveals three extreme variations. Figure 48_{9, 11} (left posterior foot of mouse 1339 and right posterior foot of mouse 1381) has very similar types, showing as they do marked atrophy of all the bones and great lateral and dorsal tortions. In Figure 48₁₀ (left posterior foot of mouse 1240) the bones of the foot are so completely absent that the leg appears to end in the tarsal bones.

SEGREGATIONS OF NORMAL STRAINS OR STRAINS WITH LOW PERCENTAGE OF ABNORMALITY

Pedigree Chart IV (Fig. 50) shows the descendants of mating ♀ 10913 *hh₂ffrp* × ♂ 10916 *hhofflp*, 21 per cent of which were abnormal. Five of the daughters of this mating were bred and all gave abnormal young. All but one mating of these young (♀ 20439 *hhlffa* × ♂ 20437 *hhlffo* — 14 per cent abnormal young)

		$\frac{10913}{hh_2ffrp}$		$\frac{10916}{hhoffp}$		$\frac{5}{1}$	
$\frac{20439}{hhffla}$	$\frac{20437}{hhffo} \frac{1}{7}$	$\frac{20441}{hhffo}$	$\frac{20437}{hhffo} \frac{8}{12}$	$\frac{20625}{hhrrfvp}$	$\frac{20678}{hhrrfra} \frac{3}{5}$	$\frac{2}{hh}$	
		$\frac{21409}{hhffla}$	$\frac{21406}{hhffp} \frac{7}{8}$	$\frac{21375}{hhrrfo}$	$\frac{21374}{hhffvp} \frac{3}{6}$	$\frac{21}{hh}$	
$\frac{21032}{hhrrfo}$	$\frac{21038}{hhoffp} \frac{9}{4}$	$\frac{21034}{hhrrfo}$	$\frac{21038}{hhoffp} \frac{9}{12}$	$\frac{21037}{hhrrfo}$	$\frac{21374}{hhoffvp} \frac{4}{10}$	$\frac{2}{hh}$	
$\frac{21536}{hhffo}$	$\frac{21537}{hhrrfo} \frac{4}{7}$	$\frac{21538}{hhrrfo}$	$\frac{21537}{hhrrfo} \frac{3}{3}$	$\frac{23862}{hhrrfla}$	$\frac{23857}{hh_2ffrala} \frac{2}{14}$	$\frac{26}{hh}$	

Fig. 50. Pedig

$\frac{20678}{hhrrffra} \frac{2}{5}$	$\frac{20627}{hh_2fflp} \frac{21060}{hhrrffo} \frac{3}{8}$	$\frac{20627}{hh_2fflp} \frac{20678}{hhrrffra} \frac{5}{9}$
$\frac{21374}{hhffrp} \frac{2}{3}$	$\frac{21377}{hhrrffo} \frac{21374}{hhffrp} \frac{1}{5}$	$\frac{20799}{hhoffo} \frac{20798}{hhffo} \frac{4}{21}$
		$\frac{21056}{hhrrffrp} \frac{21059}{hhrrfflalp} \frac{1}{5}$
$\frac{21038}{hhofflp} \frac{2}{6}$	$\frac{21991}{hhrrffra} \frac{21993}{hhrrffo} \frac{1}{2}$	$\frac{21992}{hhrrffrp} \frac{21993}{hhrrffo} \frac{4}{4}$
	$\frac{22174}{hhoffo} \frac{22175}{hhffra} \frac{9}{8}$	
$\frac{22347}{hhffo} \frac{9}{8}$	$\frac{22348}{hhoffo}$	$\frac{22347}{hhffo} \frac{1}{25}$
$\frac{23143}{hhrrffo} \frac{9}{11}$	$\frac{23167}{hhrrffo} \frac{23164}{hhrrffo} \frac{9}{9}$	$\frac{24010}{hhoffo} \frac{24008}{hhrrffo} \frac{9}{8}$
		$\frac{24011}{hhoffo} \frac{24008}{hhrrffo} \frac{1}{15}$

chart IV

gave ratios of abnormal to normal which were higher than the ratio of the parent mating. Mating ♀20441 *hhlffo* × ♂20437 *hhlffo* gave 67 per cent abnormal, and two of their young gave 87 per cent abnormal young. Another daughter (♀20626) of this mating (10913 × ♂10916) bred to a distantly related male (♂20678) gave 40 per cent abnormal young. Three of their daughters mated to their brother gave 50, 66, and 20 per cent abnormal young. A fourth daughter of the mating ♀10913 × ♂10916, female 20627 *hh₂fflp*, mated to male 20678 *hhrffra*, produced 55 per cent abnormal young.

One of these females (♀20799 *hhoffo*) mated to her brother, (♂20798 *hhlffo*), had six daughters which were mated to their siblings. Two of these (♀21032 *hhrffo* and ♀21034 *hhrffo*), mated to their brother (♂21038 *hhoffp*), gave all normal young. However, two of the normal-appearing daughters of the pair ♀21034 × ♂21038, when mated to their brother, gave 57 per cent abnormal in one instance (♀21536 *hhlffo* × ♂21537 *hhrffo*) and 100 per cent abnormal in the second (♀21538 *hhrffo* × ♂21537 *hhrffo*).

Another daughter of the mating ♀20799 × ♂20798, female 21991 *hhrffra*, mated to her brother 21993 *hhrffo*, produced 50 per cent abnormal. Two of these animals (♀22174 *hhoffo* × ♂22175 *hhlffra*) produced young, all of which were normal. In all, there have been 104 descendants of this pair (♀22174 × ♂22175), yet only two abnormal-footed individuals have appeared among them.

This pedigree shows that it is possible to segregate strains in this particular abnormal-footed race which will continue to produce a large percentage of abnormal young. It also shows in the case of descendants of female 21991 and male 21993 that strains may also be segregated which will show little or none of the foot abnormalities. However, normality in appearance of the young of any individual pair which are themselves the young of abnormal-producing parents, is not a criterion of genetic normality, as is shown by the young of mating ♀21034 × ♂21038.

If this same thing is represented graphically in Graph I (Fig. 51) where the percentage of abnormal young is represented along the ordinate and the various succeeding generations along the

abscissa, it is found that the young of this high abnormal line fluctuate between 0 and 100 per cent abnormal.

Here is seen the apparent tendency toward separation into normal and abnormal lines. Mating ♀20799 × ♂20798 produced

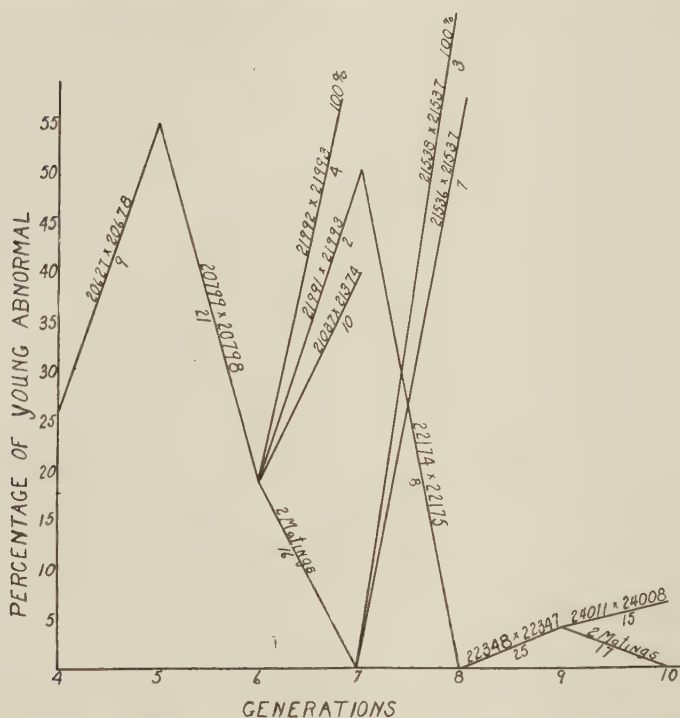


FIG. 51 (Graph I). Percentage of abnormality in succeeding generations

EXPLANATION OF FIGURES 51-55 (GRAPHS I-V)

In this and the following graphs, the numbers above the lines are the ledger numbers of the individuals involved in the matings. Below the line is the number of young which each mating produced.

five females. Two of these when bred produced young which were all apparently normal. Three other females from this same mating, when mated to their brothers, produced abnormal young in excess of the ratio of the parent mating. The young of one of these matings (♀21991 × ♂21993) when bred *inter se* (♀22174 ×

♂22175) produced young all of which were apparently normal. It is of interest to note that in both of the cases where this line tended toward normal, the normal-appearing animals produced abnormal young, in one case to the extent of 100 per cent (♀21538 × ♂21537).

When Pedigree Chart II (Fig. 42) is shown in the same way

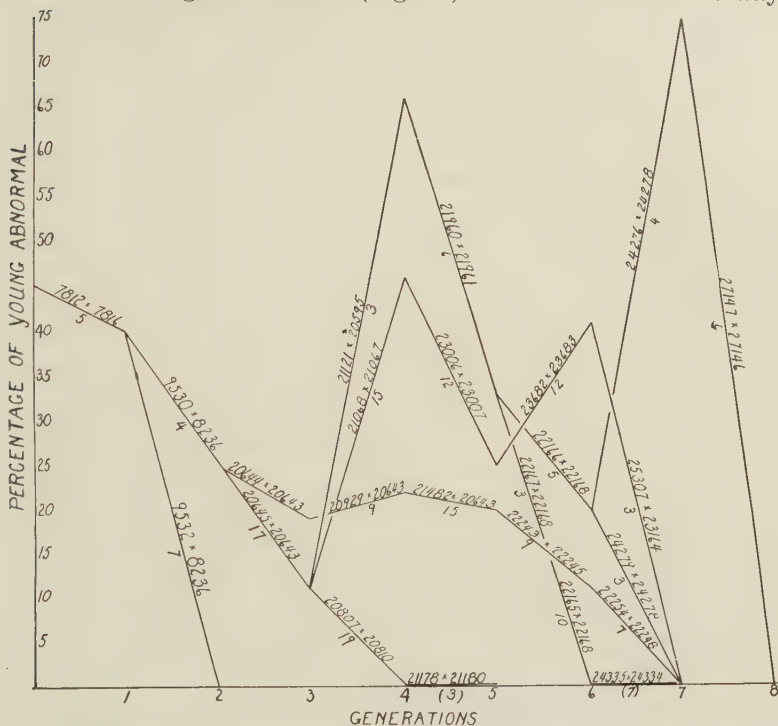


FIG. 52 (Graph II.) Percentage of abnormality in succeeding generations

(Graph II, Fig. 52), it is found that in the first seven generations three different lines segregate, all of which have a tendency toward normality. The first of these reaches normality in two generations, the second in four, and the third in seven generations. Other lines, after fluctuating three, four, or five generations between twelve and seventy-five per cent abnormality, all segregate and show a tendency toward normality.

Having reached normality in external appearance, these individuals may or may not breed true to normal; some of them reach as high as forty per cent abnormal young in two generations from normality.

In Graph III (Fig. 53) the same line of mice returns to normality three times; and in as many instances, these normal-appearing individuals gave birth to abnormal young. This same type of behavior is shown in Graph IV (Fig. 54), which is computed from Pedigree Chart II.

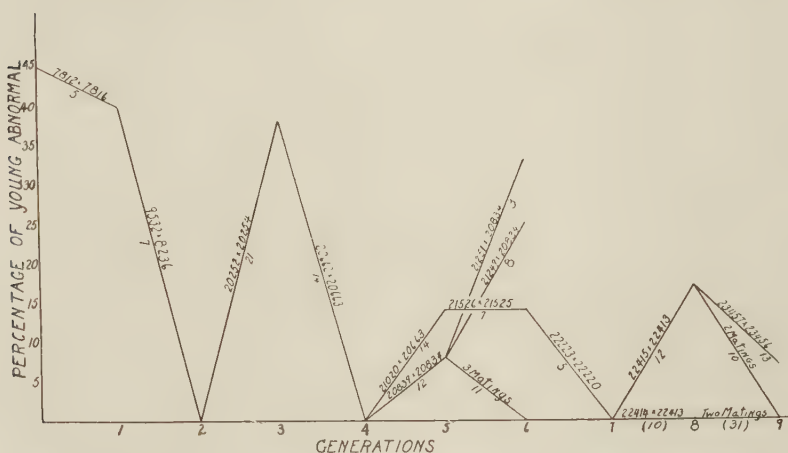


FIG. 53 (Graph III). Percentage of abnormality in succeeding generations

From these charts and graphs it is seen that inheritance of the abnormal foot defect is very irregular; that highly abnormal lines may breed true to high abnormality or may throw young all of which are normal; and that low abnormal lines may throw young which when bred will give birth to as high as 100 per cent abnormal. It will, therefore, be of interest to note the general trend of the percentage of abnormal young born in the different generations in this stock.

The mice recorded upon Pedigree Charts I, II, and III (Figs. 41-43), range from the fourth generation after the abnormality first appeared in the stock (σ 1369) to the fourteenth generation. In computing the percentage of abnormal mice in each genera-

tion (Graph V, Fig. 55), it is found that the percentage of abnormal young in the fourth generation was 33, in the fifth generation 47, in the sixth 28, in the seventh 21, in the eighth 17, in the ninth 12, in the tenth 11, in the eleventh 18, in the twelfth 9.5, in the thirteenth 13, and in the fourteenth 20.

From this curve, it is seen that the percentage of abnormality has a tendency to grow less from the sixth to the thirteenth generations. The probability is that the sharp rise of the curve at

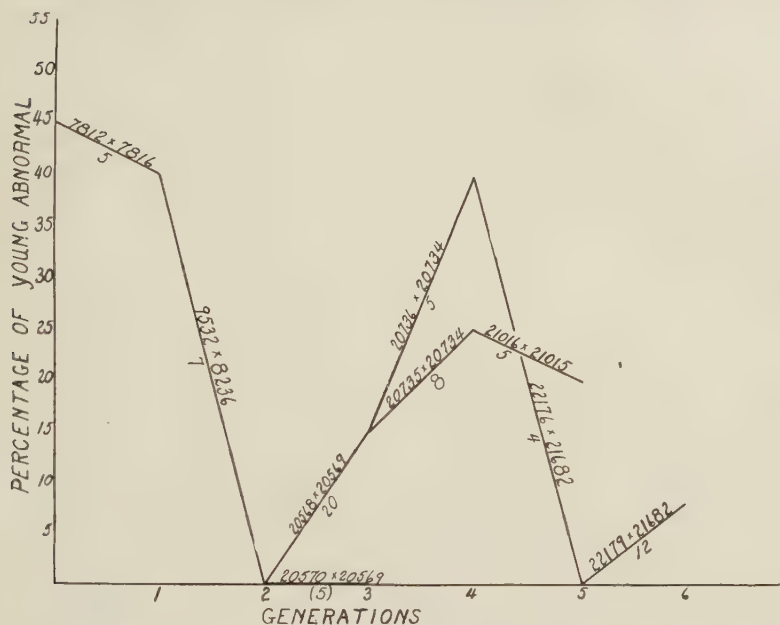


FIG. 54 (Graph IV). Percentage of abnormality in succeeding generations

the fourteenth generation is due to the smallness of the numbers involved and to the fact that the strain which has reached this generation happened to be matings which were showing a momentary high percentage of abnormality.

From this last graph (Graph V, Fig. 55), it is also seen that, while the percentage of abnormal young apparently decreases between the fourth and the ninth generations, it tends to remain at a constant level. A drop in percentage, therefore, in any given

line, does not necessarily mean that the abnormality is fading out genetically.

THE PROOF OF INHERITANCE

In Pedigree Chart I (Fig. 41) is shown male 1369, which was the first abnormal animal recorded by Little and Bagg in their original paper. This male (σ 1369), mated to his sister (φ 1367), gave 31 young, 3 of which had abnormal feet, showing in this way that either one or both of them carried the character or characters which cause the abnormality. That male 1369 carried the

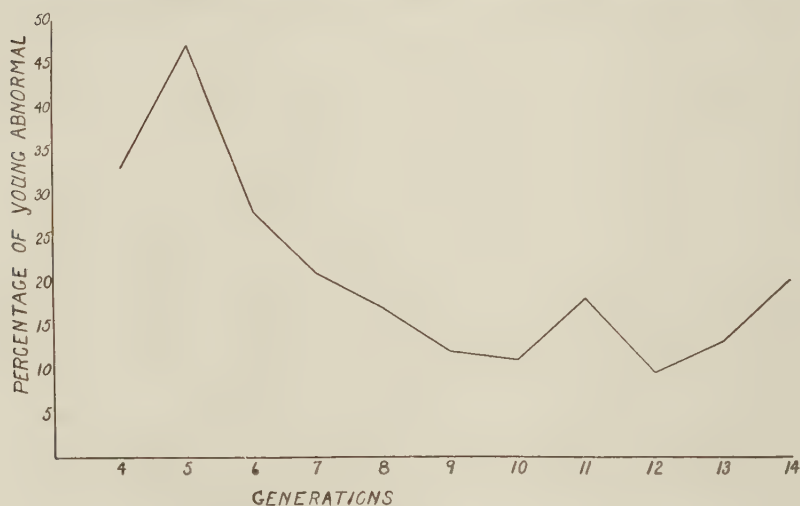


FIG. 55 (Graph V). Percentage of abnormality in succeeding generations

gene or genes for this deformity is shown by the fact that abnormal feet were found in his inbred descendants after he had been out-crossed to a normal female (Little and Bagg, 1923, 1924).

Pedigree Chart I (Fig. 41) shows the record of this out-cross. Female 1740, when mated to her brother (σ 1741), gave female 4063 *hh₂slsl*, which was the mother of the first abnormal-footed animal that appeared in this line after the out-cross.

The descent of the father of this abnormal animal is shown on the same Chart. Female 1738, mated to her brother (either σ 1739 or σ 1742), gave female 2577, which was abnormal, and

male 2579, which was normal in appearance. These two animals (♀ 2577 and ♂ 2579) had a son (♂ 4006 *hhlffo*), which when mated to female 4063, before mentioned, gave female 6340 *hhoffla* and male 6339 *hhoffabn*. After the first appearance of the abnormality following the out-cross, it appears regularly in the descendants of these mice. (See Pedigree Charts II and III, Figs. 42-43.)

TYPES OF INHERITANCE

That this abnormality behaves genetically as hypostatic to normal is demonstrated by Pedigree Chart I (Fig. 41). This conclusion becomes much more convincing, however, if the F_1 generation of an out-cross made in this laboratory is taken into consideration. In this experiment three brothers (♂ 24208 *hh₂ffrala*, ♂ 24209 *hh₂ffrala*, and ♂ 24210 *hhlffrala*) were out-crossed to normal unrelated albino females of the Bagg strain.

RECORD OF OUT-CROSS

Mating	No. of Young	No. of Litters	Mating	No. of Young	No. of Litters
36 × 24208	1	4	Brought forward	31	181
57 × 24208	2	8	58535 × 24210	1	5
58+1 × 24210	1	4	58726 × 24210	2	14
59 × 24210	1	5	58758 × 24210	2	11
69 × 24208	1	6	58759 × 24210	1	7
71 × 24208	1	9	58761 × 24210	2	11
72 × 24208	3	20	58787 × 24210	2	13
73+1 × 24208	2	14	58788 × 24210	3	20
74 × 24208	2	15	58858 × 24209	1	10
75 × 24208	4	27	58879 × 24209	2	13
76 × 24208	1	5	58880 × 24209	2	14
77 × 24208	3	13	58881 × 24209	2	12
97 × 24208	2	12	58882 × 24209	2	14
99 × 24208	1	6	58919 × 24209	2	13
58439 × 24210	2	10	58920 × 24209	3	19
58498 × 24210	2	10	58924 × 24209	3	14
58532 × 24210	2	13	58925 × 24209	2	12
			58926 × 24209	3	18
	31	181	Total	66	401

Average litter size, 6.07.

From these matings, 66 litters were obtained in which there were 401 young, an average of 6.07 young per litter. All the offspring of these matings were normal in appearance.

NORMAL OVERLAPS IN RELATION TO OTHER
KNOWN MORPHOLOGICAL VARIATIONS

Morphological abnormalities in mammals are by no means rare, since many different forms have been reported. In mice one of the best known of the deformities which occur is the defect described as "waltzing." This is a condition affecting the middle ear, making the afflicted individuals progressively deaf, and also influencing the semicircular canals in such a way that the animals lose their sense of balance and keep up a continual whirling motion. This condition has been described by Yerkes (1907), who found the character to behave as a recessive to normal and demonstrated that the F_2 waltzers show a marked extension of variability in the amount to which the character manifests itself.

A second morphological character which appears in mice is that of hairlessness and roughness of the skin. This condition has been described by Allen (1904), who found it to be a recessive. Brooke (1926) has also described this character.

A third deformity appearing in mice is that of kinky tail, described by Plate (1909), and later by Blank (1916), who discovered that the natural form of growth was distorted by inequalities in growth of the epiphyses of the caudal vertebrae. This character is inherited irregularly, two abnormals sometimes giving normal young; and normal animals extracted from such crosses sometimes give kinky-tailed individuals.

Danforth (1925) has reported a "strain of doubles" in mice: "The anomalous individuals range from 'Y' shaped specimens with four hind legs and two tails to those with relatively slight degree of doubling in the external genitalia . . . apparently connected with the same manifestation is the appearance of hemorrhagic testes, *spina bifida*, and occasional other anomalies such as microphthalmia."

Bonhote (1912) has reported the waltzing character in *Rattus rattus*. The inheritance of this character has, however, never been worked out, but it appears to be recessive and sex-linked.

In guinea pigs, Castle (1906) has described a polydactylous

race which behaves irregularly. Although it is supposedly a dominant, individuals homozygous for this character sometimes throw normal-appearing young, which in turn may have polydactylous progeny.

Wright (1916) has reported that rough coat in guinea pigs varies over a wide range in its manifestations, being influenced by modifying factors to such an extent that smooth-coated individuals derived from this stock sometimes throw rough-coated young.

As mentioned before (see page 510), variability of morphological characters brought about in mammals by external agents has been reported by Stockard and Papanicolaou, by Guyer and Smith in rabbits, and by Little and Bagg in mice. All these investigators have described eye defects accompanied by variations of physical instability, ranging from normal to such grossly abnormal defects that the animals could not survive.

In the present experiment, great variation in the amount of abnormal feet has been shown (Figs. 44-49). These abnormalities range from normal-appearing feet to almost complete absence of the extremity.

That many of the normal-appearing individuals of this strain are normal overlaps is illustrated by the following:

1. Miss A. M. Bean,⁵ while working upon the skeletal structure of the extremities of individuals in this stock, found that: (a) These abnormalities grade from slight degrees of departure from the normal types to grossly malformed members; (b) A reduction of the tarsal and carpal bones by fusion occurs in practically all the abnormal feet and in some of those otherwise apparently normal.

2. In Table I is shown the record of 106 matings of normal-appearing individuals homozygous for abnormal feet. Forty-nine of these threw abnormal young, and the young of the remaining 57 matings were normal. This shows that at least 46 per cent of the normal-appearing animals in that strain are genetically abnormal, although physically they overlap the normal condition.

⁵ Paper in press.

INADEQUACY OF EXPLANATION OF THE GENETIC BEHAVIOR
ON THE BASIS OF A SINGLE GENE

While the Pedigree Charts and the record of the out-cross matings (see page 529) prove conclusively that this deformity is definitely inherited in a hypostatic manner, the data do not show any of the simple ratios which might be expected if it were the manifestation of the action of a change from normality in a single gene.

This fact is demonstrated by the results of the back-cross matings of these same three abnormal males (σ 24208, σ 24209, σ 24210) to their daughters by the normal Bagg females (Table II).

If the expectations are computed for such a cross upon the supposition that the abnormality is a simple Mendelian recessive to a normal dominant, it might be expected that the out-cross

TABLE I

SHOWING THE DISTRIBUTION OF THREE CLASSES OF MATINGS
IN THE STOCK MICE

CLASS	Number of matings	Matings which threw abnormals	Number of litters	Number of young	Average litter size	Matings which threw normals	Number of litters	Number of young	Average litter size	TOTAL YOUNG	TOTAL LITTERS	AVERAGE LITTER SIZE
ffo × ffo	106	49	107	575	5.37	57	88	407	4.61	982	195	5.03
ffo × ffabn	68	55	95	496	5.22	13	17	86	5.05	582	112	5.19
ffabn × ffabn	42	35	67	318	4.73	7	9	40	4.44	358	76	4.69
TOTAL	216	139	269	1389	5.16	77	114	533	4.65	1922	383	5.01

TABLE II

SHOWING THE DISTRIBUTION OF THE BACK-CROSS MATINGS

CLASS	Number of matings	Matings which threw abnormals	Number of litters	Number of young	Average litter size	Matings which threw normals	Number of litters	Number of young	Average litter size	TOTAL YOUNG	TOTAL LITTERS	AVERAGE LITTER SIZE
$ffo \times ffabn$	71	51	79	579	7.3	20	25	150	6.0	729	104	7.00

represented by the formula $FF \times ff$ would produce one type of young, all of which would have the genetic constitution Ff . These animals would all be normal in appearance inasmuch as the normal gene is dominant over the gene for abnormality. This much of the expectation is borne out by fact. (See record of matings, p. 529.)

If, however, these F_1 females of the formula Ff are back-crossed to their fathers, whose genetic formulae are all ff , it might be expected that the resulting young would be of the formulae Ff and ff in equal numbers, since the mothers form two types of gametes and the fathers but one. The experimental data do not even approximate a one to one ratio.

In this cross, 71 different matings were recorded (Table II). These produced 104 litters in which were 729 young. If the deformity is dependent upon a single Mendelian factor, it should be expected from these computations that 364 of these young would have abnormal feet. In reality there were 124 abnormal individuals (Table V). This is but 17 per cent or one third of the number expected in a back-cross involving a simple recessive.

The deficiency may be attributed to one or both of two causes:

1. Many of the normal-appearing individuals may be genetically

abnormal overlaps with the normal type; 2. The homozygous individuals may be lethal.

The data compiled for the stock mice indicate that part of the deficiency in the number of observed abnormal animals may be explained by the first of these causes. Table I shows that in 106 matings of apparently normal individuals, 49 threw abnormal young, and 57 threw normals only. This would indicate that at least 46 per cent of the somatically normal-appearing animals in this stock are genetically abnormal.

Since it is impossible to obtain a pure breeding strain of these abnormalities, it may be that the homozygous recessives are lethal. If this is true, the average litter size of the back-cross matings should be larger than that of the stock matings, or of those matings following the back-cross in which it is possible to obtain that combination of genes which will produce homozygous recessive individuals.

The stock mice in this experiment produced 1922 young in 383 litters with an average litter size of 5.01. In the F_1 generation there were 66 litters in which there were 401 young, an average of 6.07 per litter. In the back-cross generation there were 729 young produced in 104 litters, with an average of 7.00 per litter. The F_2 generation, or the stock mice after the back-cross, produced 318 young in 61 litters with an average of 5.2 per litter. In the normal stock of Bagg albinos, from which the females for the out-cross were obtained, the average litter size is found to be 5.2 (1067 young in 205 litters).

It will be seen from these figures that the stock mice, the F_2 generation, and the normal Bagg stock are very similar in regard to litter size. By computing the expectation of the effect of a lethal factor in the back-cross generation upon the basis of the average litter sizes in the stock and out-cross mice, it is estimated that the back-cross should be larger than that of the stock and smaller than that of F_1 . Since the observed average litter size in the back-cross generation is 7.00, and that of F_1 is 6.07, it appears that the data do not provide supporting evidence that some of the stock abnormalities are lethal, but rather that the larger litters in the out-cross and back-cross generations

are due to the vigor of heterosis. The data are, however, not to be considered as completely conclusive against the presence of some lethal influences.⁶

IF BOTH PARENTS ARE ABNORMAL, THE PROBABILITY OF
ABNORMAL YOUNG IS INCREASED

The mice available for the compilation of data in this experiment may be divided into four classes:

1. Those matings of the stock mice following the out-cross of male 1369 to normal Bagg albinos (stock).
2. The out-cross of the three abnormal brothers (σ 24208, σ 24209, σ 24210) to the normal females.
3. The back-cross of these same males to their daughters.
4. The back-cross F_2 following this out-cross.

In the following discussion the first of these classes will be designated "stock mice," the second "out-cross," the third "back-cross," and the fourth "back-cross F_2 ."

By turning to Table III, it is found that in the stock mice there were 57 $f/o \times f/o$ matings which threw all normal young, and 49 which threw abnormals. In the back-cross F_2 stock there were 8 matings which threw only normals and 4 which threw abnormals. In the stock mice there were 13 matings of the type $f/o \times f/abn$ which threw normals and 55 which gave abnormals as compared with 20 and 51 in the back-cross stock, and 3 and 12 in the back-cross F_2 stock for matings of this type. In the matings of the type $f/abn \times f/abn$, the stock mice produced 7 matings which threw normal young and 35 matings which threw abnormal young as compared with 1 and 17 in the back-cross F_2 matings of this type. In totaling these results, it is found that in the $f/o \times f/o$ matings, 65 produced all normal young and 53 gave some abnormals. In the $f/o \times f/abn$ type of mating, 36 produced all normal young and 118 gave some abnormals. In the $f/abn \times f/abn$ type of mating, 8 threw all normal young and 52 produced some abnormals.

⁶ Intra-uterine foetal counts are being made in this stock. The data to date show that there is an average of 1.4 degenerating embryos in each mouse. The average litter size of the mice examined would have been 6.5.

TABLE III

SHOWING RATIOS OF MATINGS WHICH THREW ABNORMALS

TYPE OF MATING	A Number of matings which threw normals	B Number of matings which threw abnormals	Ratio of A to B
Stock $f\bar{f}o \times f\bar{f}o$	57	49	1 : 0.86
Back-cross $F_2 f\bar{f}o \times f\bar{f}o$	8	4	1 : 0.50
Total $f\bar{f}o \times f\bar{f}o$	65	53	1 : 0.81
Stock $f\bar{f}o \times f\bar{f}abn$	13	55	1 : 4.23
Back-cross $f\bar{f}o \times f\bar{f}abn$	20	51	1 : 2.55
Back-cross $F_2 f\bar{f}o \times f\bar{f}abn$	3	12	1 : 4.00
Total $f\bar{f}o \times f\bar{f}abn$	36	118	1 : 3.28
Stock $f\bar{f}abn \times f\bar{f}abn$	7	35	1 : 5.00
Back-cross $F_2 f\bar{f}abn \times f\bar{f}abn$	1	17	1 : 17.00
Total $f\bar{f}abn \times f\bar{f}abn$	8	52	1 : 6.50

From these data it is apparent that, when two normal-appearing animals of this stock are bred, the chance is about one to one that some of the young will have abnormal feet. If an abnormal-footed individual is bred to a normal-appearing animal of this stock, the chances of producing abnormal animals from the mating are three to one. If two abnormal-footed individuals are bred, the chances increase to six to one that the mating will produce some abnormals.

If Table IV is examined, it will be seen that 575 young were born to the 49 matings which produced abnormal young. Of these, 110 or 19.1 per cent were abnormal-footed. In the back-cross F_2 matings of this same type, 42 were born to four matings, seven, or 16.6 per cent, of the individuals were abnormal-footed. If the percentage of abnormal individuals born to animals producing abnormals in the $f\bar{f}o \times f\bar{f}abn$ type of mating is computed, it is found to be 28.4 per cent in the stock mice, 21.4 per cent in the back-cross, and in the back-cross F_2 38.0 per cent. In the matings of the $f\bar{f}abn \times f\bar{f}abn$, this percentage increases to

TABLE IV

SHOWING PERCENTAGE OF ABNORMAL YOUNG IN ABNORMAL MATINGS

TYPE OF MATING	Matings— young all normal	Matings—some abnormal young	Abnormal indi- viduals	Young born normal matings	Young born abnormal matings	Percentage of abnormal young abnormal matings
Stock <i>f</i> fo × <i>f</i> fo	57	49	110	407	575	19.1
Back-cross F ₂ <i>f</i> fo × <i>f</i> fo	8	4	7	89	42	16.6
Total <i>f</i> fo × <i>f</i> fo	65	53	117	496	617	18.9
Stock <i>f</i> fo × <i>f</i> fabn	13	55	141	86	496	28.4
Back-cross <i>f</i> fo × <i>f</i> fabn	20	51	124	150	579	21.4
Back-cross F ₂ <i>f</i> fo × <i>f</i> fabn	3	12	35	14	92	38.0
Total <i>f</i> fo × <i>f</i> fabn	36	118	300	250	1167	25.7
Stock <i>f</i> fabn × <i>f</i> fabn	7	35	105	40	318	33.0
Back-cross F ₂ <i>f</i> fabn × <i>f</i> fabn ...	1	17	43	4	77	55.8
Total <i>f</i> fabn × <i>f</i> fabn	8	52	148	44	395	37.4

33.0 in the stock mice, and to 55 in the back-cross F₂ stock. In totaling the matings of these three types in the different stocks, it is found that in the *f*fo × *f*fo type matings which produce abnormal young, 18.9 per cent of the young are abnormal. In the *f*fo × *f*fabn type this percentage increases to 25.7 and in the *f*fabn × *f*fabn type to 37.4.

Table V shows in the column to the left the number of young born in the three types of matings in the several stocks. To the right of this column is recorded the number of these young which were abnormal-footed. Reading the totals of the three types of matings, one finds that in this stock, when two normal-appearing animals are bred, 10.5 per cent of the young born are abnormal-footed. When an abnormal animal is bred to a normal-appearing individual of this strain, this percentage increases to 21.1; and when two abnormal animals are mated, 33.7 per cent of the young born are abnormal-footed.

TABLE V

SHOWING PERCENTAGE OF YOUNG WHICH HAD ABNORMAL FEET

TYPE OF MATING	Number of young born	Number of young with abnormal feet	Percentage of young which had abnormal feet
Stock $ffo \times ffo$	982	110	11.2
Back-cross $F_2 ffo \times ffo$	131	7	5.3
Total $ffo \times ffo$	1113	117	10.5
Stock $ffo \times ffabn$	582	141	24.2
Back-cross $ffo \times ffabn$	729	124	17.0
Back-cross $F_2 ffo \times ffabn$	106	35	33.0
Total $ffo \times ffabn$	1417	300	21.1
Stock $ffabn \times ffabn$	358	105	29.3
Back-cross $F_2 ffabn \times ffabn$	81	43	53.0
Total $ffabn \times ffabn$	439	148	33.7

From these data it is clear that the greater the abnormality which enters a cross, the greater is the chance that some of the young will be abnormal.

LOCALIZATION OF THE ABNORMALITIES

If this abnormality of the feet is due to a simple recessive gene, it might be expected that the frequency with which each of the four feet appears abnormal will be approximately one fourth of the total number of abnormal feet. A glance at Table VI, however, will show that this is not true. As this table shows, in the $ffo \times ffo$ matings of the stock mice, 11.9 per cent of the abnormalities were in the right anterior, 23 in the right posterior, 30.9 in the left anterior, and 34.1 in the left posterior feet. This demonstrates that in such crosses the susceptibility of the individual feet to this abnormality is not equal. It shows rather that the left side is practically twice as susceptible as the right in that 65 per cent of the abnormalities were upon the left side as against 35 on the right.

In this same stock, when the cross is of the type $ffo \times ffabn$,

22.0 per cent of the abnormal young have the deformity in the right anterior, and 19.2 in the right posterior as against 35.5 in the left anterior, and 23.1 in the left posterior feet. Here again the left side predominates over the right in the percentage of abnormalities in the ratio of 58.6 to 41.2.

When the cross was of the *ffabn* $\times$ *ffabn* type, 14.6 per cent of the abnormalities were in the right anterior foot, 28.4 in the

TABLE VI

DISTRIBUTION OF THE ABNORMALITIES

Stock	Right anterior	Left anterior	Right posterior	Left posterior	Total number of abnormal feet	Number of young
<i>ffo</i> $\times$ <i>ffo</i>	15	39	29	43	126	982
Percentage	11.9	30.9	23.0	34.1	...	
<i>ffo</i> $\times$ <i>ffabn</i>	39	63	34	41	177	582
Percentage	22.0	35.5	19.2	23.1	...	
<i>ffabn</i> $\times$ <i>ffabn</i>	19	43	37	31	130	358
Percentage	14.6	33.0	28.4	23.8	...	
TOTAL	73	145	100	115	433	1922
Percentage	16.8	33.4	23.1	26.5	...	
BACK-CROSS						
<i>ffo</i> $\times$ <i>ffabn</i>	44	76	20	32	172	729
Percentage	25.5	44.1	11.6	18.6	...	
BACK-CROSS F_2						
<i>ffo</i> $\times$ <i>ffo</i>	2	5	2	2	11	131
Percentage	18.1	45.2	18.1	18.1	...	
<i>ffo</i> $\times$ <i>ffabn</i>	17	25	3	4	49	106
Percentage	34.6	51.0	6.1	8.1	...	
<i>ffabn</i> $\times$ <i>ffabn</i>	18	26	7	10	61	81
Percentage	29.5	42.6	11.4	16.3	...	
TOTAL	37	56	12	16	121	318
Percentage	30.5	46.2	9.9	13.2	...	

right posterior, 33.0 in the left anterior, and 23.8 in the left posterior. Here, as in the crosses mentioned above, the predominance of the left over the right side is apparent, although here it is not so marked, being in the ratio of 43 per cent right to 57 per cent left. In the totals of the abnormalities occurring in the four different feet, it is found that in the stock mice as a whole 16.8 per cent of the abnormalities occur in the right anterior, 23.1 in the right posterior, 33.4 in the left anterior, and 26.5 in the left posterior feet. In the totals for right and left, it is found that 39.9 per cent of the deformities occur upon the right side and 59.9 on the left. In contrast to this, if the totals for the two front feet and the two hind feet are calculated, it is found that they approach very closely to equality, there being 50.2 per cent of the abnormal feet in the anterior extremities and 49.6 in the posterior.

In the back-cross generation (Table VI), all the matings were of the type $f\bar{f}o \times f\bar{f}abn$. Here 25.5 per cent of the abnormalities were in the right anterior, 11.6 in the right posterior, 44.1 in the left anterior, and 18.6 in the left posterior foot. In this cross the left side predominates over the right in the ratio of 62.7 per cent to 37.1 per cent. *Unlike the stock mice, the ratio of anterior abnormalities to posterior abnormalities is 69.6 per cent to 30.2 per cent.*

In the back-cross F_2 generation the number of $f\bar{f}o \times f\bar{f}o$ type of mating is small, but shows the dominance of the left anterior foot over all the rest in susceptibility to the abnormality in that it is affected in 45.2 per cent of all cases, while each of the rest is affected in 18.1. In the $f\bar{f}o \times f\bar{f}abn$ type of mating in this generation, the right anterior foot was affected in 34.6 per cent of the abnormalities, the right posterior in 6.1, the left anterior in 51.0, and the left posterior in 8.1. Here, as in the back-cross generation, the predominance of the left side over the right is shown by a ratio of 59.1 per cent to 40.7 per cent, and the predominance of the anterior part of the body over the posterior in the ratio of 85.1 per cent to 14.2 per cent.

Much the same thing is found in the $f\bar{f}abn \times f\bar{f}abn$ cross in this generation. In this type of mating, the right anterior

foot is affected in 29.5 per cent of the cases of abnormality, the right posterior in 11.4, the left anterior in 42.6, and the left posterior in 16.3. Here the ratio of abnormal left feet to abnormal right is 58.9 per cent to 40.9 per cent, and the rate of anterior to posterior abnormalities is 72.1 per cent to 27.7 per cent.

TABLE VII

PERCENTAGES OF ABNORMALITIES

TYPE OF MATING	Right feet abnormal	Left feet abnormal	Anterior feet abnormal	Posterior feet abnormal
Stock.....	39.9	59.9	50.2	49.6
Back-cross.....	57.1	62.6	69.6	30.1
Back-cross F ₂	40.4	59.4	76.7	23.1

From the totals of these three stocks (Table VII), it is found that the percentages of deformity remain very constant, 40 for the right and 60 for the left. If, however, the groupings are made for the anterior and posterior feet, it is found that there is a progression from equality in the stock mice to 76.7 per cent anterior to 23.1 per cent posterior abnormality in the back-cross F₂ mice.

INHERITANCE OF EACH FOOT IS NOT THAT OF A SINGLE GENE

Because of the variation between the stock mice and the back-cross and back-cross F₂ generations, it may be reasoned that the three brothers used in the out-cross (all of which carried two abnormal forefeet) introduced something into the out-cross in higher concentration than it occurred in the stock animals. Inasmuch as the only marked difference between the data of the stock and the out-cross animals is the increased ratio of anterior to posterior abnormalities in the back-cross and back-cross F₂ generations, it is not unreasonable to suppose that the something which these three brothers introduced was a preponderance toward abnormality in the anterior feet.

If this is true, it might be expected that, when an individual with an abnormal right anterior foot is bred to a normal-appearing animal of the abnormal stock, there will be a preponderance of abnormal right feet and of forefeet. In this experiment there were seven matings of this type. These animals produced 52 young, 21 of which were abnormal (Table VIII). Of the abnormalities, 9 were in the right anterior foot, 7 in the left anterior, 2 in the right posterior, and 3 in the left posterior. This is a ratio of 16 anterior to 5 posterior.

There were 23 matings of the type *ffo* × *ffla* (Table VIII). They produced 221 young, 61 of which were abnormal—18 having abnormal right anterior feet, 21 abnormal left anterior feet, 11 abnormal right posterior feet, and 11 abnormal left posterior feet. In totaling these numbers, it is found that 63.9 per cent of these abnormalities were in the anterior feet, and 36 per cent were in the posterior feet.

TABLE VIII

MATINGS INVOLVING ONE ABNORMAL FOOT

TYPE OF MATING	Right anterior	Left anterior	Right posterior	Left posterior	Total anterior	Total posterior	Total right	Total left	Young abnormal	TOTAL YOUNG
<i>ffo</i> × <i>ffra</i>	9	7	2	3	16	5	11	10	21	52
Percentage	42.8	33.3	9.5	14.2	76.1	23.7	52.3	47.5	..	...
<i>ffo</i> × <i>ffla</i>	18	21	11	11	39	22	29	32	61	221
Percentage	29.5	34.4	18.0	18.0	63.9	36.0	47.5	52.4	..	...
<i>ffo</i> × <i>ffrp</i>	4	17	10	12	21	22	14	29	43	117
Percentage	9.3	39.5	23.2	27.8	48.8	51.0	32.5	67.3	..	...
<i>ffo</i> × <i>fflp</i>	2	12	4	10	14	14	6	22	28	101
Percentage	7.1	42.8	14.2	35.7	49.9	49.9	21.3	78.3	..	...

There were 17 matings of the type *ffo* × *ffrp*. These animals gave birth to 117 young, 43 of which were abnormal-footed. Four of the abnormalities were in the right anterior foot, 17 in the left anterior, 10 in the right posterior, and 12 in the left posterior. In this cross 49 per cent of the abnormalities were in the anterior, and 51 per cent were in the posterior, feet.

Eleven matings of the type $f\bar{f}o \times f\bar{f}lp$ produced 101 young, 28 of which were abnormal. Of these abnormal individuals, 2 had abnormal right anterior feet, 12 had left anterior abnormalities, 4 right posterior, and 10 left posterior abnormal feet. In this cross 50 per cent of the abnormalities were in the anterior feet, and 50 per cent were in the posterior feet.

From the data (Table VI), it is seen that the four feet of the stock mice are susceptible to this abnormality in the order: left anterior, left posterior, right posterior, and right anterior. If, then, an individual bearing a single deformity is bred to an apparently normal individual, it might be expected (if these abnormalities are specific for the different feet rather than general for all feet) that the resulting progeny will vary from the normal percentage for each foot as shown by the eighth line of Table VI, and that the influence of the localization of the deformed foot will manifest itself in the ratio. Since there is a definite sequence of susceptibility to abnormality, it also might be expected that the influence of a left anterior foot in such a cross will affect the ratio more than a right anterior foot. In Table VIII, however, it is found that this is not necessarily true. It is found rather that introducing anterior abnormality into such a cross increases the percentage of anterior abnormalities, and that posterior abnormality introduced in this way tends to bring the ratio of anterior to posterior abnormality back to that of the stock animals.

Since the left side (all types of mating) is dominant over the right side, and since one left foot when it is the only abnormality in the cross does not appear to make any appreciable difference in the number of left-foot abnormals produced, Table IX, which is the record of those matings in which each parent had a left-foot abnormality, was compiled in an attempt to determine whether a double dose of "leftness" would produce young which would show a variation from the ratio of the stock mice.

TABLE IX

MATINGS IN WHICH EACH PARENT HAD A LEFT-FOOT ABNORMALITY

TYPE OF MATING	Right anterior	Left anterior	Right posterior	Left posterior	Total anterior	Total posterior	Total right	Total left	Young abnormal	TOTAL YOUNG
ffl $\times$ ffl	7	15	7	6	22	13	14	21	35	119
Percentage	20.0	42.8	20.0	17.1	62.8	37.1	40.0	59.9	..	...

This table is computed from the young of 13 matings, from which 119 progeny were obtained. There were 35 abnormal feet among these animals. Of these, 20 per cent were right anterior and 20 were right posterior, while 42.8 were left anterior and 17.1 were left posterior. In totaling these right and left, the ratio is found to be 40 per cent right to 59.9 left. The similarity between this ratio and that of the stock mice (39.8 per cent right to 60.1 left) is striking.

From these data it is evident that the inheritance of this abnormality is not specific for the four feet, but that apparently there are modifiers present, the manipulation of which changes the ratio of anterior to posterior abnormalities.

These data tend further to show that the Bagg albino females used in the out-cross introduced a modifier or modifiers which changed the localization of the abnormalities in an anterior direction.

THE RELATION OF FOOT ABNORMALITY TO EYE ABNORMALITY ⁷

The data for the abnormal-footed individuals, showing as they do departure from equality in the numbers of right and left side abnormalities, suggest the questions:

⁷ It is contemplated to make a study of the brains of a relatively large series of mice showing distinct eye abnormalities. This study is to embrace more particularly the thalamic and tectal regions to ascertain the possibility of a correlation between eye defects and receptive centers. The preparations of sections serially cut for the study of cells and fiber paths are under way.

1. Does the number of abnormal eyes occurring on the right side differ from the number occurring on the left side in this stock?

2. Does out-breeding alter this ratio in any way?

3. Does the location of an abnormal eye have any relation to the side upon which abnormal feet occur when they happen in the abnormal-eyed individuals?

The abnormal-eye data, which were derived from the same matings as those from which the abnormal-foot data were computed (Table X), show that in the 1922 young born to stock animals there were 1212 abnormal-eyed individuals. That is, 63 per cent of the young were abnormal-eyed. Of these abnormal individuals, 50.8 per cent had abnormal right eyes, 40.4 had abnormal left eyes, and 8.8 were abnormal in both the right and left eyes.

These data are in striking contrast to those of the back-cross generation. In this division of the experiment there were 729 young, 217, or 29.7 per cent, of which had abnormal eyes. This is, as was expected, almost one half of the frequency with which the abnormalities occurred in the stock mice. Of these deformities, 49.3 per cent were in the right eye, 45.6 per cent in the left eye, and 5 per cent of the young were abnormal in both eyes.

TABLE X

DISTRIBUTION OF EYE ABNORMALITIES

TYPE OF MATING	Abnormal right eye	Abnormal left eye	Abnormal both eyes	Number of young	Young with abnormal eyes	Percentage of young abnormal-eyed
Stock.....	616	490	106	1922	1212	63.0
Percentage.....	50.8	40.4	8.8			
Back-cross.....	107	99	11	729	217	29.7
Percentage.....	49.3	45.6	5.0			
Back-cross F ₂	59	56	12	318	127	39.9
Percentage.....	46.4	44.1	9.4			

It is found here that out-breeding this abnormal stock to normal females introduced something (probably the gene F), which reduced the number of abnormalities almost exactly by one half, but that the small preponderance of right-eye abnormality over left is retained in almost the same ratio in which it occurred in the stock mice.

The figures for the back-cross F_2 generation of this stock (Table X) show that the reduction in abnormalities, after having been made in the back-cross, is retained in following generations. This is not true of the foot abnormality, as has already been pointed out. In this stock there were 318 young, 127, or 39.9 per cent, of which were abnormal-eyed. This again is slightly over one half of the old stock ratio. The preponderance of right is again held in the ratio of 46.4 per cent to 44.1 per cent, which approximates that of the back-cross generation, although these figures probably mean that equality is indicated.

Of the 1922 stock mice 1212, or 63 per cent, were abnormal-eyed. Of these abnormal-eyed young, 288 also bore an abnormal foot. The distribution of these abnormal feet coincident with an abnormal eye follows, in general, the same law as that of the abnormal feet alone. That is, the right eye is abnormal simultaneously with the left feet more than with the right. This is to be expected, however, inasmuch as the abnormal feet occur in the ratio of 60 abnormal on the left side to 40 on the right. This condition is also found in the case of the left eye, where the left feet are coincidentally abnormal with the left eye more than are the right feet.

In the back-cross generation, there were 729 young, 217 of which had abnormal eyes. Of these 217 abnormal-eyed individuals, 81, or 37.3 per cent, bore an abnormal foot. Here again the left feet are more often coincidentally affected with both the right and the left eyes than are the right feet, the condition being more marked in the anterior limbs. This is to be expected since the ratio of anterior to posterior abnormal feet changes from equality in the stock mice to a 75 to 25 ratio in the back-cross generation.

In the F_2 back-cross generation, there were 318 young, 127, or 39.9 per cent, of which were abnormal-eyed. Of these 127

abnormal-eyed individuals, 80 also bore an abnormal foot. This generation diverges from the others in that the right anterior foot is coincidently abnormal with the right eye more often than is the left anterior. The customary predominance of the left abnormality holds, however, in the feet coincidently abnormal

TABLE XI

DISTRIBUTION OF FOOT AND EYE ABNORMALITIES

TYPE OF MATING	Abnormal eye coincident with abnormal foot	Right eye coincidently abnormal with				Left eye coincidently abnormal with				Number of abnormal eyes
		Right anterior	Left anterior	Right posterior	Left posterior	Right anterior	Left anterior	Right posterior	Left posterior	
Stock	288	36	38	39	53	36	50	40	33	1212
Percentage	23.7	..	..	..	..	..	..	..	..	
Back-cross	81	10	20	7	6	18	21	13	8	217
Percentage	37.3	..	..	..	..	..	..	..	..	
Back-cross F ₂	80	14	19	2	6	18	24	9	6	127
Percentage	62.9	..	..	..	..	..	..	..	..	

with the left eye.

One very striking thing about these data is the fact that in the stock mice 23.7 per cent of the abnormal-eyed individuals were coincidently abnormal in one of their feet, while in the back-cross and back-cross F₂ generation, this percentage increases to 37.3 and 62.9 per cent, respectively. This indicates that, while the actual percentage of abnormal-eyed mice has been reduced one half by out-crossing to normal females, the individuals which were abnormal-eyed in the latter two generations are increasingly likely to be abnormal in their eyes and feet coincidently.

It may be concluded from these data that:

1. There is a tendency toward equality in the number of abnormal eyes occurring upon the right and left sides in all three stocks.

2. There is apparently no causal relation between abnormal eyes on either side and the location of the coincidently abnormal feet.

3. By recovering the abnormality from the out-cross, factors favorable for abnormal eyes occurring alone have evidently been lost, and the eye and foot abnormalities in the same individuals seem in some manner to have been brought together or concentrated.

TABLE XII

SHOWING THE PERCENTAGE OF ABNORMAL-FOOTED YOUNG

TYPE OF MATING	Number young	Abnormal-footed individuals	Percentage of young with abnormal feet	Individuals abnormal in more than one foot	Percentage of abnormal individuals, abnormal in more than one foot
Stock	1922	356	18.5	68	19.1
Back-cross	729	124	17.0	40	32.2
Back-cross F ₂	318	86	27.0	32	37.2

The effect of this concentration is very marked when the case of the abnormal feet alone is considered (Table XII). In this table it is shown that of the 1922 stock animals 356, or 18.5 per cent, had abnormal feet. Of these 346 animals 68, or 19.1 per cent, were abnormal in more than one foot. The data for the back-cross show that of the 729 young 124, or 17 per cent, were abnormal. Of these 124 deformed young 40, or 32.2 per cent, had multiple abnormalities. In the back-cross F₂ generation, 86 out of 318 young were abnormal (27 per cent), and of these 32, or 37.2 per cent, were deformed in more than one foot.

It has been shown earlier in this paper that, although the character "abnormal foot" behaves as hypostatic to normal, animals homozygous for the deformity do not have 100 per cent abnormal-footed young. Of the 1922 stock animals (Table XII) 356, or 18.9 per cent, were abnormal in one or more feet. If this percentage is taken for the degree of manifestation for homozy-

gous parents, it is apparent that the back-cross generation should throw 9.2 per cent abnormal-footed young, provided the normal unrelated females had introduced nothing into the cross which affects the ratio.

From the table, however, it is seen that instead of 9.2 per cent, the abnormal animals appeared in 17 per cent of the individuals of this generation. Evidently something was introduced by the normal females which caused the deformity to make itself apparent in about twice the expected number of cases.

If, then, the ratio of abnormal to normal has been changed by out-crossing, the effect of the change should be felt in the F_2 back-cross generation when abnormal animals are chiefly selected for breeding. Table XII shows that in this generation 27 per cent of the young were abnormal. This is not quite the expectation of 34 per cent computed upon the percentage of abnormality in the back-cross generation and does not approximate 37 per cent if the expectation is computed on the basis of twice as many abnormals as appeared in the stock animals. On the other hand it is far in excess of twice the 9.2 per cent which was originally calculated for the back-cross.

From these data it may be concluded that out-crossing this abnormality introduced factors which enabled the abnormality to manifest itself in a ratio which is distinctly greater than that of the original stock.

Since both the eye and the foot defects are hypostatic to normal and since they both overlap normality at one extreme of their variation, the question arises: Are they different manifestations of the same developmental process, or are they distinct deformities and independent of each other?

In Table XIII the data for both these defects are brought together. From this it is seen that of the 1922 animals in the abnormal stock 356, or 18.5 per cent, had abnormal feet and 1212, or 63 per cent, had abnormal eyes. It is apparent from this that the conditions necessary for the manifestation of abnormal eyes are over three times as likely to occur as are these necessary for the production of abnormal feet.

Taking 18.5 per cent abnormals as the normal degree of

TABLE XIII

SUMMARY SHOWING A COMPARISON OF OBSERVED AND
EXPECTED ABNORMALITIES

	Stock		Back-cross		Back-cross F ₂	
Total number of animals	1922		729		318	
Total number abnormal-footed individuals....	356	18.5%	124	17.0%	86	27.0%
Total number abnormal-eyed individuals.....	1212	63.0%	217	29.7%	127	39.9%
Total number normal-footed individuals....	1566		605		232	
Total number normal-eyed individuals.....	710		512		191	
	Observed	Expected	Observed	Expected	Observed	Expected
Abnormal eye coincident with abnormal foot..	288	224	81	37	80	34
Abnormal eye coincident with normal foot....	924	988	136	180	47	93
Normal eye coincident with abnormal foot..	68	131	43	87	6	52
Normal eye coincident with normal foot....	642	579	469	425	185	139

occurrence of the abnormal feet in the stock strain, the number of abnormal feet which will occur may be computed for any one of the four possible combinations of these two defects, namely: (1) abnormal eye and abnormal foot; (2) abnormal eye and normal foot; (3) normal eye and abnormal foot; (4) normal eye and normal foot.

If this line of thought is followed, it might be expected that, provided the two abnormalities are different entities, 18.5 per cent of the 1212, or 224, of the abnormal-eyed individuals will also be abnormal-footed, and that 81.5 per cent of 1212, or 988, will bear the abnormal eye alone.

From the table it is seen that instead of 988 only 924 of the 1212 were abnormal in their eyes alone, but that the observed

number of abnormal eyes coincident with an abnormal foot is well in excess of the expected number — 288 as against 224.

After the same manner it may be estimated that 131 of the 710 normal-eyed young will have abnormal feet, and the 81.5 per cent of 579 will be normal in both eyes and feet. The latter type is slightly in excess of the observed number. The observed number of animals with normal eyes and abnormal feet is well below the expectation — 68 as compared with an expectation of 131.

In the data for the back-cross, it is seen that much the same sort of thing is true here also, the observed animals with abnormal eye and abnormal foot being almost twice as frequent as expected. The number of animals with normal feet in conjunction with abnormal eye is much lower than calculated.

This same condition holds true for the back-cross F_2 as well.

From these data it does not seem unreasonable to conclude that the abnormalities which occur in the feet and eyes are manifestations of the same general genetic change; namely, a developmental arrest which is operative to the greatest effect in the region of the eyes and then in the left and right feet.

This developmental arrest appears to be due primarily to the extravasation of blood in the subepithelial connective tissues of the developing foetuses. The degree to which the deformities develop depends entirely upon the age at which they start and the region in which they start. The reason for their settling largely in the eye is due probably to the prominence of this organ in development and because of its innate complexity; and any small variation from normal may well make itself evident macroscopically.⁸

CONCLUSIONS

1. Changes in the germ-plasm may be effected by X-rays.
2. These changes may be inherited.
3. The abnormalities in this particular case are hypostatic to normal conditions.

⁸ The available embryological material pertinent to these studies, fixed, sectioned, and stained after approved methods, is being accumulated with a view of making histogenetic studies of the abnormalities here discovered.

4. "Low incidence strains" may be segregated from time to time.

5. "High percentage strains" may for a time show a high percentage of abnormal animals, but all in turn throw strains which tend toward normal.

6. Having reached normal, strains do not necessarily breed true to normal.

7. The stock bred *inter se* would not therefore "lose" the abnormality.

8. There is great variation in degree of abnormality. At one end of its manifestation it overlaps normal.

9. At least 50 per cent of the genetically abnormal animals are normal in appearance. Further histological and embryological study will probably add to our information on this point.

10. The observed ratios cannot be explained on the basis of a single gene.

11. If both parents are abnormal in appearance, the probability of abnormal young is greater than if one or both are "normal overlaps."

12. The digital abnormality appears most frequently in the left anterior foot.

13. Sixty per cent of the foot abnormalities are on the left side.

14. In the "stock" mice the abnormalities are equally distributed between anterior and posterior feet.

15. Out-crossing the abnormals to normals (Bagg albino strain) increases the percentage of anterior abnormality, suggesting that modifiers for localizing the abnormality are introduced by the normal strain used.

16. Out-crossing the abnormality to normal Bagg females probably introduced plus modifiers which have increased the proportion of normal feet.

17. Sixty-three per cent of the stock animals had abnormal eyes of the type described by Little and Bagg as *hh*.

18. Eye and foot abnormalities either are due to the same general genetic change or are in some way linked together in inheritance.

19. If they are the same character, they may be different manifestations due to variation in the time of greatest influence of the gene upon the developing embryo.

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PART II: PERIPHERAL MOTOR NEURONES

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INTRODUCTION

IN PART I of this paper a study has been made of the genetic behavior of a group of abnormalities which appeared in the descendants of a race of mice, the ancestors of which were subjected to a light dose of X-rays (Little and Bagg, 1923, 1924).

One of the most outstanding of these defects is a developmental arrest which affects the feet and legs of the young of these animals, and which manifests itself in a variety of malformations that overlap the normal condition on one end of their variability, and on the other extreme produce young animals with grossly deformed paws and legs.

Since it is known that one of the more usual effects of X-ray irradiation is the alteration of the nervous system of the young (Bailey and Bagg, 1923; De Nobele and Lams, 1925), and since in development and normal function a correlation of the nervous system with the tissues of the limbs is postulated, it seemed desirable to undertake a study to ascertain whether or not the deformities of the limbs are due to improper development of the spinal cord, thus depriving the limb buds and limbs of normal innervation during their *anlagen* and developmental stages and resulting in structural defects in the adults.

Part II of this contribution records a study of the peripheral motor neurones in a series of mice which had more or less pronounced leg deformities. An investigation of peripheral sensory neurones, more particularly the dorsal root ganglia, in mice presenting paw and leg abnormalities is reserved for a later date.

MATERIAL AND METHODS

The spinal cord enclosed within the dura, together with the brain stem to the level of the midbrain and the proximal portion of the lumbo-sacral plexus, was removed from the dorsal side. The nerve roots of the several segments of the spinal cord were retained in sufficient length to enable ready recognition. The spinal cord thus removed was moistened with normal salt solution and transferred to a clean slide with the ventral aspect of the cord against the slide. The lumbo-sacral nerves were then spread fan-shape and tied to the slide by means of fine thread. By applying slight tension to the brain stem, the cord was extended in an approximately straight line. When this alignment was attained, the central end was tied to the slide by means of thread. The spinal cord attached thus, cephalad and caudad, was maintained in this position on the slide through the successive steps of histologic technique.

The cords studied were fixed in trichloroacetic-mercury-alcohol mixture (Huber, 1927), dehydrated in absolute alcohol, cleared in xylol, and permeated in paraffin of successively increasing degrees of melting point — 45°, 52°, 54°. In order to facilitate study, complete serial sections were made. Certain of the series were cut transversely, and other series were cut longitudinally in the coronal plane.

In preparing the cords which were to be cut transversely the procedure was as follows: After fixation and partial dehydration the cords were cut into equal segments of approximately one centimeter in length. These segments were then arranged on a slide in close lateral proximity with the ventral aspect of the cord against the slide, and the central end of each segment in alignment with the other several segments. In this arrangement

they were tied to the slide by several windings of a fine thread and were then carried through hydration, clearing, permeation with paraffin, and were embedded in paraffin in the same arrangement and alignment. Each stroke of the knife thus cut five or six sections, which were fixed to the slide in serial arrangement. There is by this method slight loss of tissue at each cross-section level at which the cord is cut into successive segments.

In preparing the longitudinal series, the cords were arranged on a glass slide coated with a thin film of glycerine, the ventral aspect of the cord resting upon the slide. Embedded thus in paraffin, the ventral side of the cord was presented to the knife when the paraffin block was placed in the microtome.

Both cross and longitudinally cut series were sectioned into respective series at a thickness of from 50 micra to 30 micra, the latter thickness becoming standard as the work progressed. These series were cut on the sliding microtome, the longitudinal sections embracing the entire length of the spinal cord. The sections as cut were arranged serially on slides to which they were fastened by means of albumin fixative.

After removal of the paraffin, the sections were stained in a dilute aqueous solution of toluidin blue, differentiated in aqueous solution of lithium carbonate and alcohol and mounted in balsam. Treated by this method, the large multipolar cells of the spinal grey were brought very clearly to view. The multipolar cell bodies presented a relatively deep blue coloration of the Nissl granules. Long segments of the major dendritic branches were evident in the preparations.

The longitudinally directed sections were found to be especially useful in the study of the cell bodies of the peripheral neurones. In such sections serially arranged, it was an easy matter to obtain a survey of the entire peripheral motor system of the spinal cord. Since the series of sections began on the ventral aspect of the spinal cord and of the ventral grey columns, the level at which any section passed through the grey column is readily calculable. Inasmuch as the sections in the several longitudinal series extended from the medulla to the cauda equina, there was encountered no loss of substance such as pertained to the

transversely cut cords, and all cell counts may be relied upon as giving the full number of cells.

Thirty-three spinal cords were prepared and studied. These were sectioned as shown in Table I.

Type of cord	Cut longitudinally	Cut transversely
Normal	7	2
Abnormal	5	6
Operated	8	5

The normal cords were taken from normal Bagg albino mice. This strain of mice is closely related to the abnormal strain in that abnormal males have been out-crossed upon two different occasions to females of the normal Bagg stock. The animals which were operated upon were selected from this same stock of normal Bagg albinos. All animals used were adults at the time they were killed and their spinal cords removed.

THE GREY MATTER OF THE SPINAL CORD OF MAMMALS

The spinal cord of vertebrates is readily separable into—

A) White matter, consisting of ascending and descending nerve tracts.

B) Grey matter, which is composed of —

1. The cell bodies of motor neurones with their dendrites and axones.
2. Neurones of other nerve cells, the axones of which are distributed largely within the central nervous system.
3. Axones and collaterals of axones terminating within the grey matter.
4. Sustentacular tissue, largely of ectodermal origin.

The relation which these two portions of the cord bear to each other varies greatly in the different vertebrate forms. In mammals as compared with lower vertebrates, the proportion of grey to white matter increases in favor of the white, so that one may generalize and state that the increase in the relative size of the cords in higher forms is due chiefly to the increased

number of ascending and descending fibers in the cord. If exact measurements are made, it is found that the proportions of the white and grey matter vary in different mammals, and also within different species of the same class. Measurement of the size of the animal must also be taken into account in examinations of this kind.

E. deVries (quoted from Kappers, p. 1120) in comparing the cords of smaller and larger animals states that "the white increases in the cross-section as the cube and the grey substances as the square," as the total size of the animal increases.

In all forms lower than the amphibians, the relation of grey and white matter is fairly constant throughout the cord. However, with the appearance of limbs, as in the frog, enlargements arise in those regions of the cord which send innervation to the appendages. The size and extent of these enlargements vary directly as the size and extent of the musculature which they innervate. A striking example of this is found in the reptiles, in which form there are three types of cords.

1. In the sphenedons (and crocodiles which have both trunk and extremity musculature) cervical and lumbar enlargements are well developed.

2. In the snakes, where the extremities are lacking, there are no cervical or lumbar swellings.

3. In the turtles, which have no thoracic muscles but do have musculature for the neck, tail, and extremities, the cord is strikingly thin in the thoracic region, but is well developed in the cervical and lumbar regions (Kappers, 1920).

In mammals there are wide variations in the shape of the cord which are readily and directly correlated with the development of the musculature. For instance, in the whale the lumbar enlargement is lacking, while the cervical enlargement is well developed. In the kangaroo, the greater musculature of the posterior extremities manifests itself in the larger swelling of the cord in the lumbar region.

Since these enlargements correspond to the regions where the large nerve trunks from the muscles of the extremities join the cord, it is not surprising to find that these swellings are co-

incident with the localization in the ventral grey of the motor neurone cell bodies, the axones of which form the nerve fibers making up the ventral roots of the spinal nerves.

Microscopic study of various regions of the cord reveals at once the fact that the cell bodies of the motor neurones of the spinal grey are divisible into several groups and columns, two of which are relatively constant:

1. A mesial group which occupies that region of the ventral horn adjacent to the ventral median fissure, and which extends practically throughout the entire length of the cord.

2. A lateral group which manifests itself in the cervical and lumbar enlargements, and which is located lateral to the mesial group. The addition of this group in specific and somewhat restricted regions causes the cervical and lumbar swellings.

The mesial group, extending as it does throughout the cord with the possible exception of the fifth lumbar and first sacral segments, is generally conceded to contain the cell bodies of the peripheral motor neurones, the axones of which innervate the musculature of the trunk.

The motor neurone cell groups of the lateral column of the ventral horns fall into four main cell columns or groups presented here, as outlined by Bruce in his topographic atlas of the spinal cord:

1. Ventro-lateral (4-8C; 2L-2S).
2. Dorso-lateral (3-8C; 2-5L, 1-3S).
3. Retro-postero-lateral (8C-1T; 1-3S).
4. Central (2L-2S).¹

In the study of the finer localization of the peripheral motor neurone cell bodies for the innervation of special muscles or muscle groups of the extremities, the work has been largely upon the human spinal cord. Such observations may here be grouped under the following considerations:

¹ See on this subject: Hepburn and Waterson (1904), Cunningham (1877), Waldeyer (1888), Kaiser (1891), Collins (1894), Van Gehuchten (1898, 1899), Hatschek (1896a-b), Marinesco (1892, 1898), Sano (1897, 1898), Parhon and Goldstein (1901 a, 1901 b), Bikeles (1904, 1905).

1. The cords of humans who had suffered amputations of various kinds: Van Gehuchten (1898), Sano (1897, 1898), Collins (1894), Bikeles (1904, 1905), Campbell (1896), Dickson (1873), Dreschfeld (1879), Edinger (1882), Genzmer (1876), Hayem (1873), Dejerine and Mayer (1878), Kahler and Pick (1880), Marinesco (1898), Parhon and Goldstein (1901 a and b), Taft (1920), Van Gehuchten and DeBuck (1898 a and b).

2. Studies made of the cord after death from musculature atrophy: Kanai (1914), Friedreich (1873).

3. Studies of the cords of *Abrachii*: Curtis and Helmholtz (1911), Elders (1910).

4. Experimental studies in which nerve roots were sectioned or muscle tissue removed: Coghill (1913, 1924, 1926), Shorey (1909), Detwiler (1926), Van Gehuchten (1898), Warrington (1898).

From these researches, it is evident that the degeneration changes in the cord observed by certain people (after cases of amputation) are not always confirmed by the observations of others. From the evidence available, however, it is clear that the loss of large bodies of muscle by amputation, through developmental failures as in *abrachii*, or by means of operations performed on the developing embryo, all have very definite effects upon the shape of the grey matter in the region of the cord which normally supplies the motor nerves to the muscles which are absent.

From the combined work of investigators in this field, it may be concluded (quoting in substance Kapper) that: The finer localization is such that the cells of origin for the most distal muscles are derived from the most ventral part of the perivisceral division of the myotome, the ventral division thus being supplied by the more dorsal neurones. Thus the cell group, the neuraxes of which supply *pectoralis major* (a ventral myotome derivative), lie dorso-lateral to those for *latissimus dorsi*, while the cells of origin for fibers to the arm and hand lie dorsal and lateral to those for *pectoralis major*. The position of the cells for the fingers in nucleus retro-postero-lateral 8C-1T explains the striking lateral extension of the anterior horn in the

8th C segment of primates. The localization for the lower extremities is similar to that of the upper, although they are not so pronounced and definite. A satisfactory explanation for this reversal is wanting; there is uncertainty whether it is owing to primary effects or to secondary changes due to neurobiotaxis.

From this it may be conjectured that: 1. The cell bodies of the peripheral neurones supplying the muscles associated with body statics are situated ventro-medially in order to lie in closer relation with the caudal continuation of the medial longitudinal fasciculus and with the vestibulo-spinal tract; 2. The cell bodies of the peripheral neurones innervating the muscles concerned with the highly organized activities of hand and foot are situated dorsally in close proximity to the great descending paths coming from higher centers, which govern more particularly synergic muscular action, especially through the rubro-spinal path.

These observations pertaining to the topographic relations of the cell bodies of the peripheral motor neurones of the spinal cord, as very briefly reviewed here, are in their main phase applicable to the study of the peripheral motor mechanism of the spinal cord of mammals in general and more particularly to the study of the higher mammals. Such differences in detail as may be revealed by special study of the spinal cord of any mammalian form may be correlated more or less definitely with specialized development of the muscular system.

THE SPINAL CORD OF THE MOUSE

To facilitate the portrayal of the spinal cord of the mouse and more particularly the numbers and topographic relations of the cell bodies of the peripheral motor neurones as found in the ventral grey columns, there are shown here a number of figures sketched at uniform magnification and drawn from cross-cut and longitudinally cut series. In these sketches the white matter and the grey matter are merely outlined, emphasis being given to the motor cells found in the grey matter of the respective sections. An attempt has been made to represent accurately the number of cells falling to each section. To facilitate figure clearness, however, slight latitude was taken with reference to cell size in the

hope that in the reduction necessary for figure reproduction the cells sketched should not be lost to view.

The cross-section series, Figures 56 and 57, are drawn from a series of cross-sections of the spinal cord of the mouse cut at a thickness of 30 micra, and representing levels approximately one millimeter apart. In making this statement cognizance is not taken of the fact that there is a slight loss of tissue in cutting the cord into several segments, as was done in this procedure. That with this method the loss of tissue is appreciable in a spinal cord like that of the mouse can be ascertained by computing the number of sections in the series and multiplying the same by the section thickness. This product, when compared with the length of an uncut cord, shows the tissue lost. However, the figures chosen for the several sketches are in sufficiently close approximation to enable the observer to discern the form and relation of the ventral and dorsal horns of the grey matter at successive levels and to enable comparison of the relative proportions of the grey and white matter at the various levels. Of the several sketches in Figures 56 and 57 sketches 1 to 8 fall to the cervical region, sketches 9 to 21 to the thoracic region, and sketches 22 to 30 to the lumbo-sacral region.

When Figures 56 and 57 are examined, it is seen that, beginning with sketch 1, the mesial groups are well marked, and that they continue throughout the length of the cord, being present in every level sketched. This is in marked contrast to the cells of the lateral columns which begin in the first and extend through the eight sketches of the cervical region. These cell groups are not found in the sections through the thoracic region, but reappear in the twenty-second section and continue to the last level shown.

In certain of the cervical sections the cell groupings described for humans are well marked. For example, in the fifth sketch, in addition to the dorso and ventro-mesial groups, the ventro-lateral, dorso-lateral, and post-dorso-lateral groups are well marked. If the series is examined above and below this section (fifth sketch), it will be seen that the lateral groups begin in the first section and gradually increase to the fifth section, below which they gradually diminish until in the ninth section they have disappeared, and the

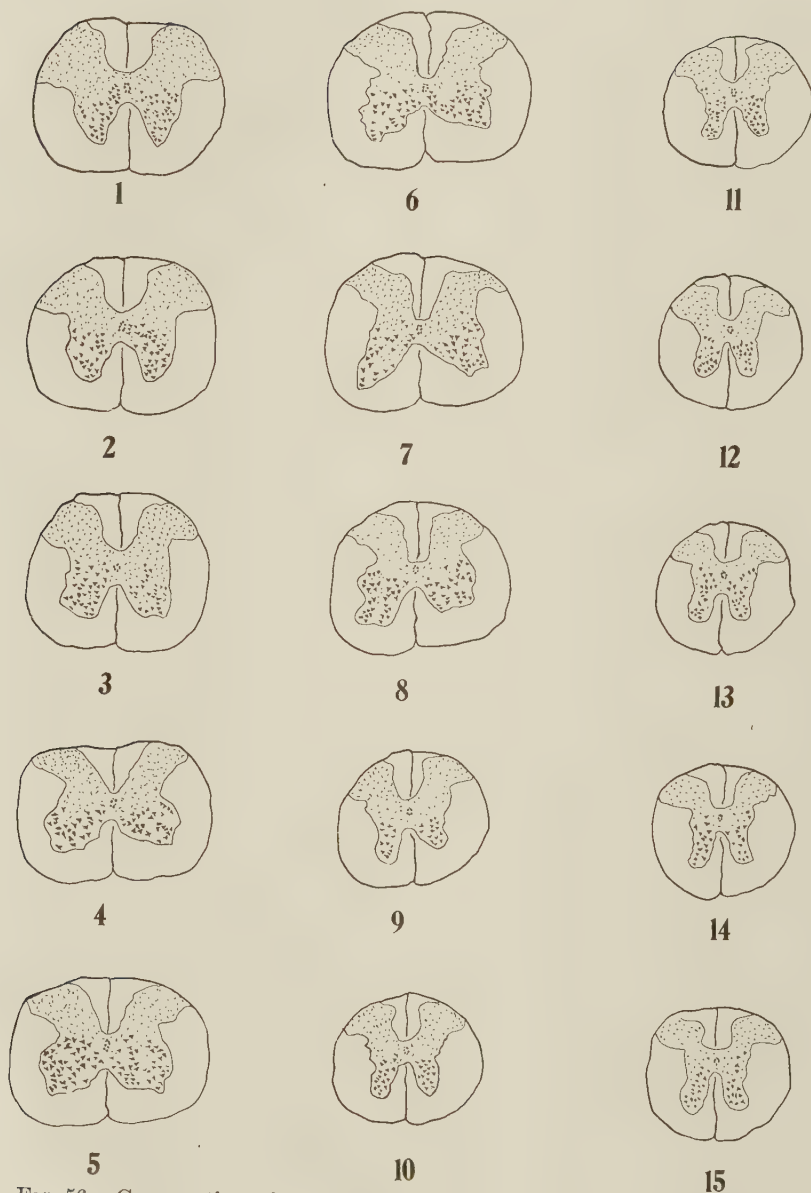
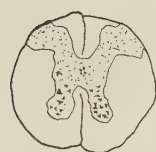


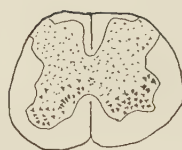
FIG. 56. Cross-section of the spinal cord of the mouse at one-millimeter intervals



16



21



26



17



22



27



18



23



28



19



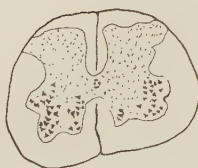
24



29



20



25



30

FIG. 57. Cross-section of the spinal cord of the mouse at one-millimeter intervals

cord takes on the contours of the thoracic region. These sections also demonstrate the impossibility of judging the number of cells on the two sides of the cord by counting those appearing in any one section. This is also true when cell columns are studied, since a group may be well marked on one side and entirely absent on the other.

Sections 9 to 21 inclusive show the relative uniformity of the thoracic region. Here the mesial cell groups are the only ones which are present.

Beginning with the twenty-second section and extending through the last section sketched, the lateral columns again appear, grow larger, and diminish. Here, as in the cervical enlargement, the cell groups vary on the two sides of any one section, being generally more closely grouped and less distinguishable than the nerve cell columns in the cervical region.

Figure 58 presents a drawing of nearly the entire length of the spinal cord of the mouse, portrayed in four successive segments. The drawings were made from longitudinally cut series, the sections of which were cut at a thickness of 30 micra. The series was cut by beginning ventrally. Twenty sections of this series contain practically all the cell bodies of the peripheral motor neurones of the spinal cord. The entire left column of the figure and nearly all the right column were drawn from one section, while the middle columns were drawn from an adjacent section. The several parts of Figure 58, when thought of as forming a continuous figure, show very clearly the ventral grey columns bordered by the white matter of the respective halves. They portray clearly the cervical and lumbar enlargements of the ventral grey columns consequent to the great increase in the number of the motor neurones destined to innervate the musculature of the extremities. There is no loss of substance in sections of longitudinal series, since they extend without interruption from the medulla to the conus medularis. In the longitudinally cut series an attempt was made to cut the two sides of the cord symmetrically. Experience has shown this to be difficult. Only a slight rotation of the cord on embedding in paraffin (so that the grey column in one half of the cord is met 3 or 4 sections of 30 micra thickness before that found in the other



FIG. 58. Coronal section through the ventral cell columns of the spinal cord of the mouse

half is met) makes an appreciable difference in the symmetry of the two sides — assuming that the ventral grey columns of the two sides of the spinal cord are symmetrical. Even such slight difference in the symmetry of the two sides makes it difficult to judge accurately in any one section the comparative number of the motor cells in the two ventral grey columns. However, a slight want of symmetry of the ventral grey columns on the two sides can only be regarded as desirable in making a total count of the motor neurones. Because of this discrepancy in the number of cells on the two sides of the cord in any one section, a comparison of the numbers found is not possible until all the motor cells of a segment have been counted.

MOTOR NEURONE CELL COUNT

In an attempt to determine whether or not the deformed musculature of abnormal-footed animals is correlated in any way with the motor portion of the spinal cord, cell counts were made of the motor nerve cells in the ventral horns of the spinal cords of a number of mice.

The first counted were taken from normal Bagg albino stock. These animals were from the same stock as were the females to which the abnormal-footed males had been out-crossed on two previous occasions. In order to establish a norm, seven of these cords were counted. All these mice were adults when killed, but no selection was made to obtain animals of uniform size.

The cell counts were made from serial longitudinal sections of the cords which had been prepared as recorded in Section 2.

The method of counting used was as follows: Since the cords were cut from the ventral side, the sections lay upon the slides with the right side toward the counter's left. A thin sheet of isinglass, across which lines had been scratched at 2 mm. intervals, was laid upon the slide and the first line adjusted (under the microscope) to lie over some definite portion of the cervical end of the cord which remained constant throughout the series of sections. After the isinglass had been put in position, the right and left sides of the cord were counted separately by 2 mm. segments. The records of the counts made were tabulated upon an especially pre-

pared sheet which was ruled to simulate a cord across which the isinglass graduate had been placed.

Ordinarily all the motor cells were contained in the first 25 or 30 sections of a series. Sometimes, however, the cord had been rotated slightly in embedding in the paraffin, so that all the sections of a series had to be examined.

By counting in this manner, it was seldom that the cell counts on both sides of the ventral median longitudinal fissure were the same in the several divisions of a 2 mm. segment or in the section as a whole. It was only upon adding the totals for the different sections that a similarity in the number of cells contained within the 2 mm. segments on opposite sides of the ventral fissure, or of the two horns taken as entities, became apparent.

Table I represents the average cell count of the seven normal cords which were studied.

It will be seen from this table that the parts of these cords which contain motor nerve cells in the ventral horns were approximately 48 mm. in length. The most striking thing about the count is the closeness with which the number of cells in the 2 mm. segments on the two sides of the median fissure approximate each other. More striking still are the totals for the two sides, which show a difference of only 69 cells.

From this table it is seen that such a cell count corresponds very closely to the general contour of the spinal grey. The cells begin rather abruptly in the cervical region, and rapidly grow more numerous until the fifth 2 mm. segment is reached. They then diminish rapidly until, at a distance of 4 mm. from this segment, the count reaches the number characteristic of the thoracic region.

The number of cells in this region increases slowly as the segments proceed caudally until in the lumbo-sacral swelling (17th 2 mm. segment) there are over 1,000 cells in a 2 mm. length of cord. Caudal to this section, the number of cells diminishes rapidly until at the end of the 24th 2 mm. segment they disappear.

From this average of the counts of several cords is obtained a typical cord which may be taken to represent that of a normal mouse of this stock. It may be said to contain 13,751 motor nerve

TABLE I
AVERAGE OF SEVEN NORMAL CORDS

Left cervical	28	32	Right cervical
	293	286	
	431	489	
	597	606	
	751	771	
2,567	467	467	2,651
Thoracic	247	232	Thoracic
	201	213	
	195	197	
	201	208	
	236	234	
1,642	273	266	
Lumbo-sacral	289	299	1,649
	335	321	
	391	361	
	469	449	
	507	530	
2,087	387	420	2,081
Coccygeal	201	177	Coccygeal
	156	161	
	111	91	
	55	71	
	18	28	
545	4	1	529
TOTAL	6841	6910	

cells, 6,910 of which are on the right side, and 6,841 on the left side of the cord, or approximately 7,000 peripheral motor neurones in the ventral grey column on each side of the spinal cord of the mouse.

This uniformity of the two sides also holds true for larger segments of the cord, as is shown by dividing the Table into cervical, thoracic, lumbo-sacral, and coccygeal segments. In this manner it is found that there are approximately 5,218 cells in the cervical

region, 3,291 in the thoracic region, 4,168 in the lumbo-sacral region, and 1,074 in the coccygeal region.

Small errors may enter into this distribution of cells owing to one or both of two variables:

1. Inconstancy in placing the isinglass graduate so that the number of cells in the first segment will always be approximately the same.

2. Variation in length of the cords which may distort the number of cells appearing in any one segment. (This is especially true of the lumbo-sacral and coccygeal regions.)

It will be seen that, since the cell count of this average normal cord is regular on its two sides, any variation in the cell count of cords of mice which had abnormal feet would be readily detected, since the nerve supply to any one limb will have its motor nerve cells on one side of either the cervical or lumbo-sacral sections as mapped out on the normal cord.

THE ABNORMAL CORD

Five abnormal cords were counted:

	Right side	Left side
1. 1618 hhlffla	7139	7284
2. 1507 hhlffrala	7420	7397
3. 1515 hhoffrala	7463	7596
4. 1240 hhrfflalp	5874	6016
5. 1235 hhrffla	7512	7242

The following symbols are used to describe the deformities of the abnormal animals:

1. *hh* for the head or eye abnormality.
2. *ff* for the foot deformity.
3. *s sl* for the saddled condition of the back.

In this manner *hhrffralp* is used to designate an animal which was abnormal in its right eye as well as in its right anterior and left posterior feet. In some of the early records *ff₂a* was used to designate individuals which were abnormal in both forefeet.²

² No counts were made of the motor cells of cross-section series because of the loss of tissue in cutting the cord into segments.

Inasmuch as the cord of 1507 *hhlffrala* was of the same length as the average normal cord, it will be used as an illustration in comparing the abnormal cord with the normal.

TABLE II
1507 *hhlffrala*

Left cervical	142	142	Right cervical
	405	348	
	580	523	
	712	642	
	819	837	
3,059	401	420	2,912
Thoracic	217	179	Thoracic
	186	164	
	188	189	
	186	156	
	204	200	
	164	177	
	219	247	
1,635	271	294	1,606
Lumbo-sacral	321	319	Lumbo-sacral
	370	433	
	521	597	
	628	635	
2,110	270	282	2,266
Coccygeal	133	138	Coccygeal
	147	132	
	167	180	
	123	136	
616	46	27	613
TOTAL	7,420	7,397	

Since both forefeet of this animal were abnormal, it might be expected that (if there is any difference in the motor innervation to an abnormal foot and to a normal foot) both sides of the cord in the cervical region would show a deficiency of cells when compared with the cervical region of the average normal cord, and

that the other three sections would correspond closely to the homologous segments of the normal cord.

Upon examination the cervical region of the cord of mouse 1,507 was found to contain 5,971 cells as compared with 5,218 cells in the same region of the normal cord. The other three regions were seen to correspond even more closely, there being 8,846 cells in the lower three sections of the abnormal as compared with 8,533 cells in the same regions of the normal cord.

From these data it is doubtful if there is any deficiency of motor nerve supply to the abnormal feet of this stock.

TABLE III

COMPOSITE OF FIVE ABNORMAL CORDS

Left cervical	81	81	Right cervical
	363	366	
	510	526	
	684	633	
	756	760	
2,811	417	450	2,816
Thoracic	199	200	Thoracic
	177	187	
	186	199	
	185	204	
	206	214	
1,389	195	224	1,492
	241	264	
	311	280	
	332	355	
	545	525	
2,294	645	588	2,214
	461	466	
	227	249	
	147	161	
	119	82	
Coccygeal	59	60	Coccygeal
	26	27	
	9	6	
587			585
TOTAL	7,081	7,107	

If a composite cord is made of the counts of the five cords of abnormal mice studied (Table III), it is at once seen that the left side of the cervical region should show the effect of the abnormalities in the left anterior foot of all these mice.

The similarity of the totals of each of the four segments on the two sides of the cord is very striking. When they are compared with the average of the seven normal cords, it is again apparent that there is no deficiency of cells in any one of the four segments or upon either side of the cord.

It will be seen from this table that in any individual cord the closeness with which the cell counts for the two sides of any .2 mm. segment are almost always nearer the number opposite them than they are to the count of any of the other segments.

Wide variations from this general finding are attributable to these facts:

1. The cell columns on the two sides of the cord are not directly opposite each other on the two sides of the cord.
2. Inaccuracy in placing the isinglass squarely across the cord.
3. Inaccuracies in counting.

Usually in cases where there are temporary variations from approximate equality, the deviations are compensated for either just above or immediately below the regions which do not balance numerically.

OPERATED MICE

After it became evident that there was no appreciable and constant difference in the motor cell count of the spinal cords of normal mice and those of mice with leg deformities, it was thought to be pertinent, in the interests of accuracy, to endeavor to determine the precise location of the cell bodies of the motor neurones innervating the musculature of the upper and lower extremities. With this in view nerves of the extremities were severed in the hope of obtaining tigrolysis in the cell bodies of the injured neurones. In certain mice the sciatic on the one side was severed, in others the median and ulnar of one side at the level of the elbow, and in still others the left paw was amputated at the wrist.

It was thought that, should constant and evident tigrolysis obtain in certain groups of motor cells of the lateral columns, a differential count could be made and more careful scrutiny given to such regions of the lateral columns of motor cells of the cervical and lumbo-sacral portions of the spinal cord in the mice with leg deformities. In case cell destruction had obtained, a noticeable difference in the cell count of the affected areas would be noticeable. Such results would indicate the regions of the cords of abnormal animals which could with profit be studied more closely for cell count deficiencies.

Fourteen mice were operated upon under complete anesthesia. Eight had the left sciatic nerve isolated, cut, and a segment removed; three had their ulnar and median nerves severed at the elbow and a segment removed; and three had the left paw amputated at the wrist. Seven of the animals were killed twenty-two days after the operation; four were killed twenty-six days after the nerves were cut; and the three remaining were allowed to live thirty-seven days after the nerves were severed.

The cord of mouse 25 was injured and made useless in the

TABLE IV

LIST OF MICE OPERATED ON

Mouse	Sex	Date operated	Date killed	Time in days	Nerve cut	Sectioned
21	♂	Oct. 9	Oct. 31	22	left sciatic	cross
22	♂	Oct. 9	Oct. 31	22	left sciatic	longitudinal
23	♀	Oct. 9	Oct. 31	22	left sciatic	longitudinal
24	♂	Oct. 9	Nov. 4	26	left sciatic	longitudinal
25	♀	Oct. 9	Nov. 4	26	left sciatic	cord destroyed
26	♂	Oct. 9	Nov. 4	26	left sciatic	cross
27	♂	Oct. 9	Nov. 15	37	left sciatic	longitudinal
28	♂	Oct. 9	Nov. 4	26	left sciatic	cross
31	♂	Oct. 9	Oct. 31	22	left wrist	longitudinal
32	♂	Oct. 9	Nov. 15	37	ulnar and median	cross
33	♂	Oct. 9	Oct. 31	22	ulnar and median	longitudinal
34	♀	Oct. 9	Oct. 31	22	ulnar and median	longitudinal
35	♀	Oct. 9	Nov. 15	37	left wrist	cross
36	♀	Oct. 9	Oct. 31	22	left wrist	longitudinal

process of removing it from the spinal column. Of the remaining thirteen cords, eight were sectioned longitudinally in the coronal plane; five were cut in cross-sections, care being taken to cut longitudinal and cross-cut series in presenting different phases of the experimental series of mice.

The results in this series of observations were disappointing in that definite and conclusive evidence of tigrolysis was not obtained in any one of the thirteen series studied.

The spinal cords in these series were removed as in other series and fixed in the trichloroacetic-mercury-alcohol mixture. They were then embedded in paraffin, sectioned, and stained in toluidin blue solution, as described for the other series herein considered. The fixation of the tissue as concerns the cell bodies of the peripheral motor neurones and their stainability in toluidin blue leaves nothing to be desired. In the large motor cells of the respective regions of the spinal cord, where no tigrolysis was anticipated, the coloring of the tigroid or Nissl substance is evident, although it does not appear to be of distinctly granular form when fixed and stained by this method. In those regions of the cervical and lumbosacral cord where tigrolysis was anticipated in the respective series, cells appeared here and there which showed less distinctive staining. Such cells were quite as likely to have a heterolateral position with reference to the operated nerve as to have a homolateral position. Therefore, the deductions to be made on the evidence of tigrolysis in these cords are inconclusive. The evidence pertaining to motor cell destruction consequent to degeneration of the peripheral motor nerves as possibly evidenced by deficiency in motor cell count needs yet to be considered and is here presented in summarized form.

Table V presents the average cell count of the eight longitudinally sectioned cords of this series.

It might be expected, if there is any deficiency of cells due to their being missed in the cell counts because of cell destruction following nerve injury, that the count of the left side would be noticeably lower than the count of the right side, since all the nerves were severed on the left side. A glance at the totals, 6,865 for the left side and 6,968 for the right, shows that this is not the case.

TABLE V

COMPOSITE OF EIGHT OPERATED CORDS

Left cervical	11	11	Right cervical
	240	227	
	394	397	
	588	594	
	627	640	
	641	659	
2,501			2,528
Thoracic	275	286	Thoracic
	200	206	
	188	200	
	190	186	
	197	199	
	205	222	
1,772	238	238	1,826
	279	289	
	309	304	
	425	425	
	573	571	
	503	493	
2,136	326	303	2,096
Coccygeal	149	155	Coccygeal
	135	135	
	95	113	
	40	74	
	31	34	
	6	6	
456	0	1	518
TOTAL	6,865	6,968	

Since four of the cords were associated with degeneration of the left sciatic, it might also be expected that the left lumbo-sacral region would show a deficiency. This is not confirmed by the data, nor is there any marked deficiency in the left cervical region, which is the site of the motor cells of the four cords with nerve degeneration in the left fore limbs.

It may be concluded, therefore, that there is no apparent cell destruction in cords of this series in which degeneration of one or several peripheral nerves was present.

If the average of the count of the motor cells in the eight longitudinally cut spinal cords of this series is compared with the standard cord for normal mice, and the average of the five spinal cords in abnormal mice, the similarity in number of motor nerve

TABLE VI

NORMAL CORD — AVERAGE OF TWENTY CORDS

Left cervical	34	36	Right cervical
	290	282	
	436	461	
	615	608	
	702	716	
2,601	524	540	2,643
Thoracic	246	246	Thoracic
	195	204	
	190	198	
	193	198	
	213	215	
	227	238	
	257	266	
1,827	306	299	1,864
Lumbo-sacral	343	337	Lumbo-sacral
	470	458	
	568	561	
	452	460	
2,090	257	245	2,061
Coccygeal	151	159	Coccygeal
	123	106	
	72	85	
	29	46	
	16	15	
393	2	3	414
TOTAL	6,911	6,982	

cells in the different segments, both for 2 mm. segments and regional, is striking.

From these data it is evident that there is no marked difference between the cell counts of any pair of the three different types of cord (normal, abnormal, and operated). If, then, the average cord is computed for these twenty cords, a normal cord will be obtained in which the probable error of any mistakes in counting will be minimized. Such a cord is represented in Table VI, which gives the average of 20 cords: 7 normal, 5 abnormal, and 8 operated.

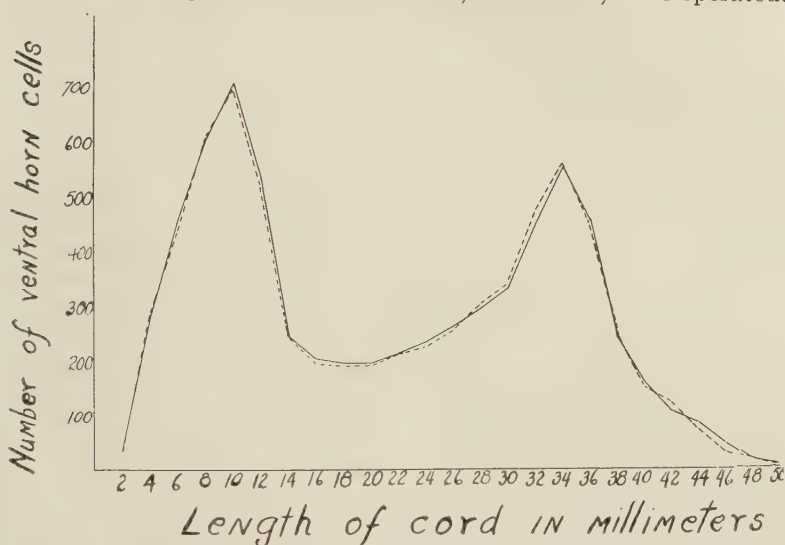


FIG. 59 (Graph I). Curve showing variations in cell counts on the two sides of the cord

If this is compared with the average cords of the normal, the abnormal, and operated mice, it will be seen that there is no marked variation in the corresponding 2 mm. segments, and that the four regional graduations show a similarity which is even more marked.

DISCUSSION OF RESULTS

In the preceding sections an attempt has been made to show, by comparing the motor neurone cell counts of normal mouse cords with similar counts of cords of abnormal-footed animals and also

with the cords of animals systematically mutilated to produce tigrolysis in motor cells of the spinal cord, that there might possibly be some deep-seated nervous defect which lay at the root of the abnormal development underlying the foot abnormalities. The findings have been entirely negative in this respect.

It has been possible, however, to compute from the data accumulated what is apparently a close approximation of the cell bodies of peripheral motor neurones in the spinal cord of the mouse (Table VI).

Graph I (Fig. 59), which tabulates the curve of the cell counts for the two sides of this normal cord, shows a remarkable case of bilateral symmetry in a mammal.

That this curve is not entirely correct, but is weighted slightly by inaccuracies in the technique of counting, is shown by Graph II (Fig. 60), which demonstrates the effects produced by inaccuracies in placing the graduate, and the effects of variability in length of different cords.

The most striking thing brought out by this study is the fact that, although the number of cells in any cord is relatively evenly distributed in the right and left ventral horns, it may vary through wide limits in the total cell count. It is thought that this fact may be attributed to the age of the mouse and possibly to the length of the cord itself (although this latter circumstance is not always true).

These findings provide direct evidence in support of those of Detwiler (1926), who found in his experiments with *Ambystoma* that the motor cell counts of the two sides of the cord were closely correlated; and that the addition of more muscle fibers by grafting, without providing in any way for the nervous innervation other than that supplied by the host of the grafted tissue, did not change the cell count in the ventral horns of the host, although in some cases the nerves were supplying almost twice as much muscle tissue as they would normally have done had there been no grafted muscle tissue.

Coghill (1913), in his work upon the development of the nervous and myotomic systems in *Ambystoma*, found that the neurones which establish the earliest contact with the cells of the myotome

are the neurones of the motor cells in the ventral horns of the cord. He also found that there is a distinct correlation between the outgrowth of the ventral horn neurones and the differentiation of the myotome; and that the outgrowth of the ventral neurone is

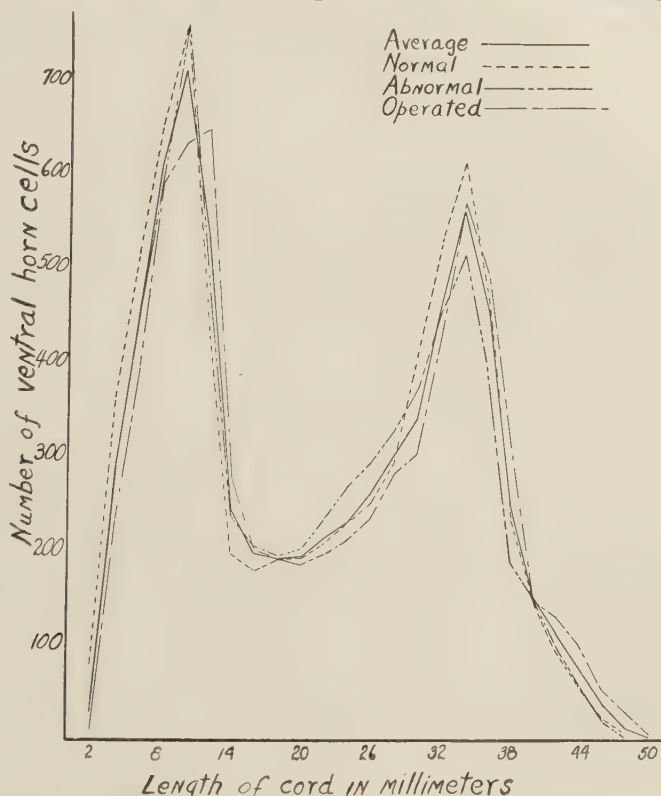


FIG. 60 (Graph II). Curves showing variations in cell counts of various types of cords

coincident with polarization of the muscle cell by the localization of pigment granules in its center.

In addition to this, Coghill concludes that the primary and most fundamental divisions of the vertebrate nervous system are longitudinal and are differentiated upon the basis of unity of function within each division.

From these observations it is evident that, as long as there is any myotomic development at all, there will be a coincident out-growth of motor neurones from the cord.

In 1909, Shorey, working on the developing chick, amputated the limb buds at an early developmental stage and showed that the non-development of large muscles has an effect upon the cord, but that when any of the muscle remained after the operation, the part of the nerve bundle which grew out to this muscle was considerably out of proportion to the size of the muscle.

It may be inferred from this that in the case of the abnormal feet, the deficiency, if there is any in motor innervation, is not sufficiently large to make itself apparent in the cell count of the cord.

CONCLUSION

We have seen that:

1. Detwiler found no change in the number of motor cells of nerve bundles which supplied more than their normal quota of muscle;

2. Shorey found remnants of muscles to be innervated by nerves which are reduced but little, if any, from their normal size;

3. Coghill found that the smallest myotomic development is accompanied by an approximately normal growth of the peripheral nerve;

4. Our own data show that the relatively small atrophy of muscle in the feet of these abnormal mice is not accompanied by an appreciable deficiency in the cord.

It may be concluded, therefore, that the underlying causes of the leg deformity in the series of mice under investigation is not to be sought in an injury to the spinal motor neurones due to X-ray irradiation.

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